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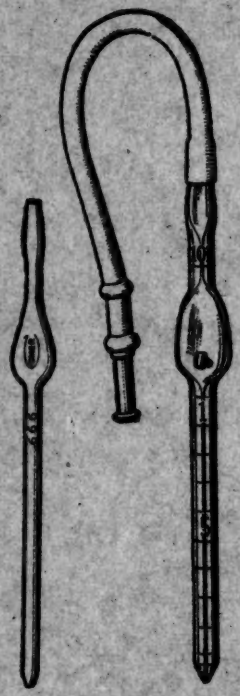

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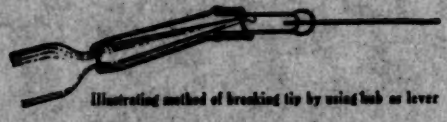

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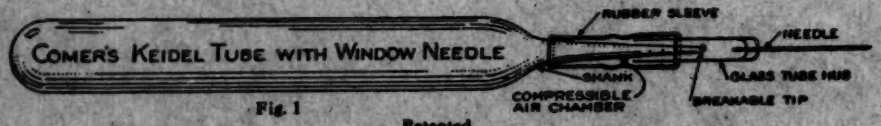

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CLINICAL AND EXPERIMENTAL

A CRITICAL ANALYSIS OF THE LYON BILE DRAINAGE TECHNIC AS AN AID TO BACTERIOLOGIC DIAGNOSIS*

WITH A REPORT ON 105 CASES

BY RALPH W. NAUSS, M.D., MICHAEL LAKE, M.D., AND JOHN C. TORREY, PH.D.,
NEW YORK, N. Y.

SINCE Meltzer in 1917[1] announced his theory regarding "The disturbances of the law of contrary innervation as a pathogenic factor in the diseases of the bile ducts and gall-bladder" and Vincent Lyon of Philadelphia in 1919[2] published the results of investigations along lines inspired by Meltzer's article, much interest has been manifested in the question of drainage of the gall bladder. Many clinicians and investigators have contributed to the rather considerable literature on this important topic. Two opposing schools of belief have arisen as to whether or not bile empties normally or can be induced to flow from the gall bladder out through the cystic and common ducts by the various means employed. Lyon and his numerous collaborators contend that such does occur normally at intervals and artificially when the proper technic is employed, while others, notably Sweet,[3] Halpert,[4] Dernel and Brummelkamp[5] hold a more or less opposite view. It is not, however, the purpose of this paper to take a definite stand in this controversy, although for the purposes of discussion we are assuming that the "B" bile is of cystic origin. Our immediate interest lies rather in the possibilities which this so-called nonsurgical drainage of the bile-ducts and gall bladder may have to offer in the field of applied clinical bacteriology.

Whipple[6] appears to be the first to have published noteworthy studies centering around the bacteriology of Lyon's bile fractions. In his article, which was published in 1921, he offers data on 25 cases in which the gall blad-

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ders were subsequently removed and similar bacteriologic examinations of their contents and walls made. In about 50 per cent of these 25 cases, he found one or more varieties of bacteria, occurring in the preoperative duodenal bile, to be likewise present in the gall bladder bile or gall bladder tissue removed at time of operation. He, moreover, admits: "In some of these (drainage biles), however, contaminations are suggested by the variety of type of organism." He regards the presence of B. subtilis, Streptococcus salivarius and Micrococcus catarrhalis as evidences of contamination in the duodenal cultures but considers the colon bacillus, hemolytic streptococcus and Staphylococcus aureus as etiologic factors, especially, if present in the "B" fraction following $MgSO_4$ instillation. He further states as his "impression from a study of these cases as well as some one hundred and fifty operative cases in which gall bladder bile and tissue from the gall bladder were cultured, that the colon bacillus is the most persistent of the bacteria found in the common duct." Rehfus and Lyon according to Whipple[6] noted this type of persistent infection and advise autogenous vaccine therapy. Likewise other investigators, notably Kelly,[7] as early as 1906 reported a high percentage of B. coli recovered from the gall bladder contents after operation.

Lyon and Kolmer in the former's book[8] published in 1923, discussed in Chapter 19 the cultural methods applicable to bile specimens recovered by duodenobiliary drainage and reported on the bacteriologic findings for a series of 64 cases. They made cultures of A, B and C bile in both hormone agar and hormone broth, employing for the latter a special flask apparatus designed to reduce the chances of extraneous contamination to a minimum. Of this series approximately 45 per cent yielded sterile biles or obvious contaminations as shown by the plate cultures, 30 per cent were positive for B. coli and 22 per cent for Staphylococcus aureus. The broth cultures gave 16 per cent positives for streptococcus. They reported the recovered streptococci as generally virulent for rabbits and also an enhanced virulence for some of the B. coli and Staphylococcus aureus strains. They considered the recovery of any of the above organisms of probable etiologic significance and as suitable for specific therapy.

About the same time as Lyon's book appeared, Piersol and Bockus[9] published an article entitled "A study of the bile obtained by nonsurgical biliary drainage, with especial reference to its bacteriology." They, however, report their bacteriologic findings in but five cases stating that "in the earlier portion of our work so much difficulty was experienced with the bacteriologic technic that the results obtained were too unreliable for permanent record." Staphylococcus albus or aureus was found most frequently with streptococcus and B. coli less often.

Boardman in an article[10] published in 1924 presented comparative bacteriologic findings in a series of 56 cases. He believes it impossible to sterilize the mouth and stomach and employed a less elaborate technic in regard to sterilization than did Lyon. He took a swab from the tonsillar area, followed by thorough rinsing of the mouth with a 1 to 5,000 permanganate solution before having the patient swallow the duodenal tube. After extracting the stomach contents, this organ was rinsed with a 1 to 10,000 permanganate

solution until the washings returned clear. When the tube appeared to be in the duodenum, its position was confirmed by fluoroscopic examination after which a culture of the duodenal contents was made. Then sterile $MgSO_4$ solution (25-30 per cent) was introduced and the "B" and "C" bile portions collected from the drainage tube and cultured. No details are given regarding bacteriologic methods or technic.

Out of his 56 cases Boardman found that the fasting stomach contents, duodenal contents, "B" and "C" bile fractions gave growths similar to those obtained from the tonsil in from 46 to 69 per cent of cases and that the various bile samples gave cultures resembling those of either mouth or stomach in 74 to 86 per cent of cases. He concludes that no reliance can be placed on the bacteriologic findings in the various bile fractions, since only in 14 to 26 per cent of the bile cultures was there an absence of organisms similar to those found in the mouth and stomach. He gives no details as to media employed but states that "the various types of staphylococci and streptococci were the organisms most frequently found."

Prior to and since the time the duodenal tube was devised and the technic of its use perfected, numerous investigators have published results of studies concerning pathologic conditions in the bile, gall bladder wall and associated structures. A considerable number of these have recently been presented in tabular review by Branch.[11] According to this table the highest percentage of positive bacteriologic growths (47 per cent) came from cultures of the gall bladder wall, while only 22 per cent of bile so examined gave positive results. In those cases listed separately as acute and chronic, 71 per cent of the former gave positive cultures but only 32 per cent of the latter proved to be positive upon culturing. Approximately 13 per cent of gall stones cultivated gave positive results.

Finally the bacterial flora of the small intestine under both normal and pathologic conditions should be considered in the evaluation and interpretation of bacteriologic findings for the bile. Bienstock[12] in 1884 called attention to the fact that the upper part of the small intestine of man contains relatively few bacteria as compared with the lower part, as well as the whole of the large intestine. This observation has been amply confirmed and our knowledge of the flora of the duodenum and lower parts of the small intestine in health and disease has been greatly extended through the use of the duodenal tube, first employed for this purpose with infants by Hess[13] in 1912 and by MacNeal and Chase[14] on adults in 1913. Van der Reis[15] in 1922 reported on a series of 350 cases and came to the conclusion that the obligate flora of the upper small intestine consisted of glycophyllic gram-positive lanceolate diplococci, B. acidophilus types and occasional gram-negative bacilli of the aerogenes group. In the middle part of the small intestine, larger numbers of bacteria were found with the gram-positive cocci decreasing and the colon group increasing as lower levels were reached. Many other workers have contributed to our present knowledge of the normal and pathologic flora of the small intestine. For a concise and comprehensive review of such work up to 1924 reference is made to an article by Goldman,[16] in which she reports her own extensive observations.

The majority of investigators ascribe the paucity of bacteria in the upper small intestine to the germicidal or bacteriostatic effect of the gastric HCl acting as a barrier in the stomach (MacNeal and Chase,[14] 1913, Hoefert,[17] 1921, Gorke,[18] 1922, Van der Reis,[15] 1922) or as repeated waves of acidity normally passing through the small intestine (Arnold and Brody,[19] 1927). On the other hand Rolly and Leibmeister[20] (1905) considered the important agent to be the healthy intact intestinal mucosa, Bogendorfer[21] (1925) certain substances ("bacteriostanius") which could be extracted from the duodenal mucosa and Lowenberg[22] (1926) the secretions of the normal duodenal mucosa. Kendall, Day, Walker and Hauer[23] (1927) in a bacteriologic and chemical study of the duodenal contents obtained with a duodenal tube from 50 cases, apparently normal as far as the duodenum was concerned, reported that 52 per cent gave little or no growth in culture media. The remainder varied from very moderate growth yielding bacteria that were chemically inert except for acid production, to heavy growths suggestive of what might be anticipated for specimens from the lower levels of the small intestine. The authors do not state whether or not the percentages of HCl in the gastric sections of these cases were normal. Recently Ricen, Sears and Downing[24] (1928) have reported on duodenal findings in 15 cases in which there was a normal or above normal percentage of gastric HCl. In 8 of these no growths were obtained from the material collected by tube and in 7 only moulds or yeasts. On the other hand, from 30 cases of achlorhydria, similarly examined on a fasting stomach, positive cultures were obtained in each case: B. coli from 10, streptococci from 24 (nonhemolytic 22 strains, hemolytic 7 strains), Staphylococcus albus or aureus from 9 and occasionally Pneumococcus, *B. alkaligenes*, yeasts and diphtheroids. These findings are, of course, in confirmation and extension of the reports of other investigators on the duodenal flora in achlorhydria, particularly as associated with pernicious anemia. Bogendorfer[21] (1922), Gorke[18] (1922), Seyderhelm[25] (1923), Goldman[16] (1924), Julich[26] (1925), Olivet[27] (1926), Knott[28] (1927), Hoefert[17] (1921) and Gorke[18] (1922) found large numbers and varieties of bacteria in the duodenum of patients suffering from gall tract disease. Julich[26] (1925) states that disease of the duodenum or gall bladder seems to be a predisposing factor in the upward growth of bacteria in the small intestine but admits that gastric achylia may be one of the causes. On the other hand Raue[29] (1924) reports that cases of cholecystitis and cholangitis may show heavy growths of B. coli in the duodenal region in spite of abnormally high gastric free acid.

PURPOSE AND SCOPE OF THE STUDY

In view of the conflicting opinions relative to the significance of cultures recovered by the Lyon duodeno-biliary drainage technic from the A, B and C bile fractions and the probable employment of such cultures for specific vaccine therapy purposes it was thought worth while to carry out a critical bacteriologic study of bile specimens so obtained in the gastroenterology department of the Cornell Clinic, New York City. The majority of the specimens were obtained by one of us (M. Lake) but for some of the later material we are greatly indebted to Dr. J. H. Whaley. The 105 cases of Tables I and

II represent patients who had been referred to this department of the clinic for diagnosis, some of them being incipient and early cases of apparent involvement of the upper right abdominal quadrant in which gall bladder pathology was thought to play a rôle and others had been diagnosed as chronic cholecystitis. From the preceding discussion of the literature, it would seem important in estimating the value of the Lyon bile drainage technic as a means of bacteriologic study of gall bladder or bile duct infection to give due consideration to the factors which in themselves promote an unusual bacterial growth in the duodenum. In previous studies we believe that insufficient attention has been given to this point. We have, accordingly, grouped our cases under the several headings of normal gastric acidity, hyperacidity, hypoacidity and anacidity. We endeavored also to secure repeated bile specimens from as many cases as possible. Further, a comparative study was made in a series of 5 cases of the bacterial content of the duodenum and that of the "B" bile fraction to determine the likelihood of the contamination of the drained bile by the duodenal flora.

BACTERIOLOGIC METHODS AND DRAINAGE TECHNIC EMPLOYED

At the clinic the special bacteriologic apparatus and methods advocated by Lyon and Kolmer[8] were employed. The former consisted of a Richardson culture flask which is prepared and used as follows: a suitable sized Erlenmeyer flask (300 c.c. capacity) is fitted with a two-hole soft rubber stopper containing inlet and outlet right angle glass tubes arranged so that the one projects somewhat farther into the flask than the other, the longer not intruding much over 1 inch. This flask is dry sterilized, the exposed ends of inlet and outlet tubes having been plugged with cotton-wool. The sterile broth to be used is then poured into this sterile flask, 100 c.c. being ample, and the sterile stopper replaced securely and firmly wired into position with suitable soft copper wire. As additional insurance of sterility, the filled flasks were placed in an Arnold sterilizer for ten minutes and after removal, cooling and drying, the cotton plugs in the inlet and outlet tubes were paraffined. In addition to these culture flasks, the clinicians were also kept supplied with sterile 20 c.c. cotton plugged test tubes for collecting 3 to 5 c.c. of bile for subsequent seeding on various media.

The procedure prior to attempts at drainage of bile was briefly as follows:

1. Patients were instructed to present themselves at the clinic at 9 A.M. on a twelve-hour fasting stomach following a motor test-meal, having also been cautioned to brush the teeth thoroughly on this morning.

2. At the clinic they were directed to gargle and rinse the throat and mouth thoroughly with an antiseptic solution somewhat similar in composition to "Lavoris" (a proprietary preparation) followed by sterile water.

3. A freshly sterilized duodenal tube was then swallowed and permitted to pass to the greater curvature of the stomach.

4. The fasting gastric residuum was extracted by gravity or by syringe aspiration.

5. The stomach was then thoroughly washed with sterile water, antiseptic solution and sterile water until the returning fluid was clear.

6. Four ounces of sterile water were then introduced through the tube in order to supply the stomach with a fluid to be emptied into the duodenum and thus encourage gastric peristalsis to assist the tube into the duodenum.

7. After securing proper placement of the tube, as shown by fluoroscope, the duodenal contents were carefully removed, observing definitely prescribed precautions, and set aside for study.

8. The gall tracts were then stimulated to evacuation of their fluid contents by instilling into the duodenum through the duodenal tube 75 c.c. of a 33 per cent volumetric solution (16.66 parts by weight) of magnesium sulphate at a warm temperature. This solution is allowed to enter by gravity through the barrel of a syringe. The end of the tube is pinched to hold its siphonage and then attached to a drainage bottle. Suction by bulb or syringe is not applied unless the fluid does not run out under gravity siphonage (under a pressure head of 18 to 24 inches) and then only by a one ounce bulb.

9. When bile begins to flow past the "window" in the tube, the collecting bottle is changed and subsequent alterations in the gross appearance of the bile are followed also by changing the collecting bottle.

If no evidence of bile has been noted in washings from the stomach or duodenum under (5) or (7), then a change from light colored to a darker colored more concentrated bile is claimed by Lyon and his followers to indicate gall bladder bile, otherwise designated as "B" bile. This fraction is the one which was usually submitted to us for bacteriologic study. (The "A" fraction is that which occupied the common and to some extent perhaps the cystic and hepatic ducts, whereas the "C" fraction is said to represent bile having its immediate source in the liver cells without much opportunity for subsequent changes to take place before collection.)

The Richardson flask was seeded by removing the paraffined cotton plugs from the inlet and outlet tubes and connecting the more deeply projecting one with the distal end (beyond window) of the duodenal tube in the same manner as for the collecting bottles, sterile preparations having been made and similar precautions observed throughout. When thus connected twenty drops of the "B" bile fraction were allowed to enter this flask, upon completion of which the distal end of the duodenal tube was slipped off the inlet tube of the culture flask and then 3 to 5 c.c. of the same fraction collected by dripping it into a sterile test tube, sterile precautions being likewise observed.

Upon receipt of this material at the laboratory, the seeded culture flask was immediately placed in the incubator and the specimen to be used for seeding other media in the refrigerator if not examined at once. A film from the latter was made to be gram-stained and examined microscopically. The broth used in the culture flask and also the plating nutrient agar were made according to the well-known "hormone" formula devised by Huntoon[30] and employed by Kolmer in his work with Lyon.[8]

Our primary cultures, then, from each drainage of the "B" bile fraction as submitted from the clinic consisted of:

1. One hundred c.c. of 1 per cent glucose hormone broth (P_H 7.4-7.6) in a Richardson culture flask seeded at the clinic at time of drainage with 20 drops of the "B" bile fraction direct from the drainage tube.

2. One or more bromcresol purple lactose agar plates P_H 7.4 surface seeded from a 4 mm. loop of the "B" bile fraction.

3. One or more glucose blood agar plates (P_H 7.4) surface seeded from a 4 mm. loop of the "B" bile fraction.

4. Cooked-meat medium (P_H 7.4) seeded usually with 0.5 to 1 c.c. of the "B" bile fraction and vaseline sealed.

5. Hormone 1.5 per cent agar containing 1 per cent glucose (P_H 7.4-7.6) for poured plates for quantitative bacterial counts.

After eighteen to twenty-four hours' incubation at 37° C. all cultures were inspected. Bromcresol purple lactose agar and glucose blood agar plates were surface seeded from the hormone broth flask, if any evidence of bacterial growth was observable in the latter. If not, the flask was returned to the incubator and examined again the following day. The surface seeded plates, if showing growth, were carefully examined for discreet colonies, of which the various types were fished and subcultured into 0.5 per cent hormone agar tubes preparatory to morphologic and differential study on sugar and other media. The anaerobic cultures (cooked meat broth tubes) were examined morphologically as soon as definite growth changes were evident, using sterile Pasteur pipettes to penetrate the vaseline seals. At the same time a bromcresol purple lactose agar plate was surface seeded from each and also a stab culture made into a 0.5 per cent hormone agar tube. If gas formation was at all pronounced, a milk tube was seeded and vaseline capped for detection of B. welchii.

Isolated streptococcus cultures were seeded into an ox-gall bile medium (dried ox-gall 5 per cent, peptone 1 per cent, and glucose 1 per cent) to test ability to grow in a bile environment. They were also planted into differential carbohydrate media, gelatin and other media necessary for identification. Selected B. coli strains were inoculated intraperitoneally into white mice as a test for virulence.

BACTERIOLOGIC FINDINGS IN THE "B" BILE FRACTIONS

In Table I is given a summary of the quantitative and qualitative bacteriologic findings, together with other relevant data, in the 109 examinations on 100 cases upon which this report is principally based. Primary subdivision is made upon the basis of the acidity found to exist in the stomach at a time prior to securing the specimen of bile through the duodenal tube. The groupings in this respect are as follows: normal, hypo-, an-, hyperacidity and unclassifiable for lack of a satisfactory gastric analysis. Thirty-nine or slightly over one-third of the biles from these 100 cases gave negative results bacteriologically, no growth being obtained in any of the culture media employed. Of the remaining 61 cases, 40 gave total bacterial colony counts ranging from a few hundred upward. These latter are subdivided into two subgroups, depending on whether or not the total count, on hormone agar plates was below or above 1,000 colonies per c.c. of the undiluted

TABLE I

BACTERIOLOGIC AND CLINICAL DATA FOR BILE SPECIMENS OBTAINED BY THE LYON TECHNIC IN 100 CASES

ACIDITY GASTRIC CONTENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION a. Unstained b. Stained (Gram)	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS a. Plate isolations b. Flask isolations	CLINICAL FINDINGS a. Clinical diagnosis b. Graham test
	NEG.	<100	<1000	>1000						
	10	9	4	6						
Normal: free, 20-40; total, 40-60			*Case* 21		5/10/27	M 55	a. Normal b. G+ cocci	600	a. S. subacidus b. G+ bacilli	a. Neg. gastrointestinal tract b. Normal gall bladder
			48		12/ 2/27	F 55	a. Evidence of stones, no "B" portion, no pus b. G+ diplococcus and thick rods	300	a. Neg. b. Neg.	a. Neg. gastrointestinal tract b. No gall bladder shadow (gall bladder removed 1½ yr. ago but no stones found)
			52		1/ 6/28	M 36	a. Mucus and epithelial pigment b. G+ chains of fused cocci	700	a. Neg. b. Neg.	a. Cholecystitis b. Neg.
				Case 76	6/ 8/28	F 54	a. No definite "B" fraction; contains evidence of biliary tract disease b. Large and small g+ diplococcus, yeast cells	100	a. Micrococcus tetragenus b. G– spore-bearing rods	a. Chronic cholecystitis b. Suggests pathology gall bladder
				Case 34	9/16/27	F 51	a. Normal b. G+ cocci, g+ rods	10,000	a. S. albus b. S. albus	a. Neg. gastrointestinal tract b. Normal gall bladder
			I	62	3/ 7/28	F 55	a. Few cholesterin crystals b. G+ chain cocci; g– rods, yeasts	650,000	a. B. coli communis, Strept. mitis b. B. coli communis	a. Neg. gastrointestinal tract (physical examination and history suggest gall bladder disease) b. Neg.
				62	4/27/28	F 55	a. Suggests cholecystitis b. G+ diplococcus, g– rods	1,500,000	a. B. aerogenes b. B. aerogenes	
				62	9/14/28	F 55	a. Suggests cholecystitis b. G– rods	1,750,000	a. Gram– bacilli (coliform) b. B. coli communis	

TABLE I—CONT'D

ACIDITY GASTRIC CONTENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION a. Unstained b. Stained (Gram)	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS a. Plate isolations b. Flask isolations	CLINICAL FINDINGS a. Clinical diagnosis b. Graham test
	NEG.	<100	<1000	>1000						
	10	9	4	6						
Normal: free, 20-40; total, 40-60—Cont'd				70	4/20/28		a. None made b. Large g+ diplobac.	62,500	a. S. salivarius b. S. salivarius	a. Chronic disease of gall bladder b. None made
			II	92	11/ 7/28	F 40	a. No "B" portion—many cells b. Masses of g+ bacteria and pigment	250	a. Saprophytic coccus b. Saprophytic coccus, S. fecalis*	a. Chronic cholecystitis, possible stones b. Suggests gall bladder pathology with stones
				92	11/16/28	F 40	a. As for Nov. 7 b. As for Nov. 7	32,500	a. Saprophytic coccus, S. mitis b. Saprophytic coccus, S. mitis	Small gall bladder containing stones removed at operation
			III	100	12/14/28	F 47	a. Doubtful "B" fraction; pus and epith. cells b. G+ diplobacillus and mycelia	58,500	a. B. acidi lactici, g+ cocco-bac. b. G– bacilli (coliform)	a. Chronic disease of gall bladder b. None made
				100	1/ 4/29	F 47	a. Doubtful "B" portion, many crystals b. G+ diplococcus	750,000	a. B. acidi lactici b. B. acidi lactici	

*No growth in 5 per cent bile, peptone broth.

TABLE I—CONT'D

ACIDITY GASTRIC CONTENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION a. Unstained b. Stained (Gram)	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS a. Plate isolations b. Flask isolations	CLINICAL FINDINGS a. Clinical diagnosis b. Graham test
	NEG.	<100	<1000	>1000						
	10	3	4	6						
Hypoacidity: free, 5 or less-15; total, 15-35			*Case* 1		2/ 1/27	F 49	a. Mucus, bile-stained pus cells; cocci and bacillinum.	100	a. Saprophytic cocci b. Streptococcus (alpha prime), S. mitis	a. Cholecystitis b. Neg. fluoroscopy shows visceroptosis
		IV	13		3/26/27	F 50	a. Bile salts, lipoid material, epithelium b. No bacteria	100	a. Streptococcus (alpha) b. Aerobic spore-bearing bacillus	a. Cholecystitis (mild) b. Suggests cholecystitis
			13		9/ 8/27	F 50	Same	None made	a. Subtiloid type b. Subtiloid type	
			47		11/30/27	F 30	a. Cholesterin crystals b. No bacteria	450	a. Neg. b. Atypical B. coli	a. Cholecystitis with stones (definite attacks colic) b. Shows laminated stones
			55		2/ 1/28	F 50	a. Numerous round cells; exfoliative duodenitis b. No bacteria	450	a. Streptococcus (alpha prime), S. equinus b. Streptococcus (alpha prime), S. equinus	a. Chronic cholecystitis exfoliatic duodenitis b. Suggests pathology gall bladder
				Case 10	3/15/27	F 37	a. None made b. G+ pleomorph. diplococcus, g+ tetracoccus	1,500	a. S. ignavus b. S. mitis and equinus	a. Marked visceroptosis b. Normal gall bladder
				26	5/26/27	F 50	a. Bile-stained pus, mucus, short columnar cells; choledochitis b. No bacteria	5,700	a. S. mitis and salivarius b. S. salivarius	a. Cholecystitis with stones b. Shows stones (operation showed gall bladder filled with stones)

TABLE I—CONT'D

CONTENT ACIDITY GASTRIC	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION a. Unstained b. Stained (Gram)	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS a. Plate isolations b. Flask isolations	CLINICAL FINDINGS a. Clinical diagnosis b. Graham test
	NEG.	<100	<1000	>1000						
	10	3	4	6						
Hypoacidity: free, 5 or less-15; total, 15-35—Cont'd				30	6/21/27	F 42	a. Bile-stained epithelia, few crystals, clumped pigment b. G+ coccobacillus and large g– bac.	7,000	a. Large nonhemolytic g+ Staphylococcus b. S. albus	
			V	30	6/21/27	F 42	a. "C" bile; same findings as above	4,500	a. S. salivarius b. S. mitis	a. Cholecystitis b. Suggests pathology gall bladder with stones
				30	10/28/27	F 42	a. True "B" bile-cholesterin crystals b. No bacteria	300	a. S. mitis b. S. mitis	
				86	10/17/28	F 55	a. No "B" bile; bile yellow suggesting cystic duct obstruction b. No bacteria	2,500	a. Yeast, atyp. staphylococcus (hemolytic) b. S. salivarius*	a. Cholecystitis b. Suggests pathology gall bladder
				94	11/16/28	F 45	a. No "B" bile, evidence of stasis in yellow bile b. G+ cocci	650,000	a. S. fecalis, B. acidilactici b. S. fecalis, streptococcus (gamma type)	a. Cholecystitis (gall bladder removed 3 yr. before showed stone in com. duct) b. None made
				107	1/25/29	F 47	a. No evidence of gall bladder disease b. G+ chain cocci	6,600	a. S. fecalis* and salivarius b. S. albus	a. Possible cholecystitis (marked visceroptosis) b. Neg.

*No growth in 5 per cent bile, peptone broth.

TABLE I—CONT'D

ACIDITY GASTRIC CONTENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION a. Unstained b. Stained (Gram)	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS a. Plate isolations b. Flask isolations	CLINICAL FINDINGS a. Clinical diagnosis b. Graham test
	NEG.	<100	<1000	>1000						
	3	2	3	4						
Anacidity: free, 0; total, 5-10			*Case* 2		2/ 1/27	M 62	a. Bile-stained epithelia and pus cells, few crystals, clumps of cocci b. None made	350	a. B. paracoli b. B. paracoli	a. Cholecystitis (jaundiced liver enlarged) b. Suggests pathology gall bladder
			98		12/ 7/28	M 52	a. Clumps pigment, few bile-stained epithelia and pus b. No bacteria	150	a. S. salivarius* b. S. salivarius, B. acidi-lactici	a. Possible gall bladder disease, visceroptosi; (fluoroscopy shows adhesions of duod.) b. None made
			121		4/24/29	M 54	a. Evidence of stasis and infection of biliary tract b. Few g+ thick bact.	500	a. S. mitis b. S. albus	a. Anacidity b. None made
				Case 4	2/15/27	M 53	a. No "B" fraction; bile-stained epithelia and pus b. G– rods; chain coccus	3,000	a. B. aerogenes b. B. aerogenes, B. proteus	a. Cholecystitis b. Suggests pathology gall bladder
				44	11/16/27	F 51	a. Cholest. crystals, bile-stained epithelia and pus cells, masses of bile pigment b. G+ pleomorph. rods	30,000	a. Atyp. B. coli b. Atyp. B. coli	a. Cholelithrasis b. Suggests small stones (2 tests)
			VI	54	1/18/28	M 55	a. Giardia +++ in duod. fluid and bile, many crystals b. Large g+ diploc., g– oval bac.	1,000,000	a. B. coli anerogenes b. Chain streptococcus (nonhemolytic)	a. Chr. cholecystitis b. Neg.
				54	10/24/28	M 55	a. Stasis with stones, not much infection b. G+ short chain coccus	250,000	a. B. coli communis b. G+ rods, enterococcus	
				54	3/27/29	M 55	a. Same findings as above b. Med. g– rods	300,000	a. B. acidi-lactici, saprophytic cocci b. G– coliform bact.	
				113	3/ 8/29	F 41	a. Evidence of stasis and mild infect.-calcium crystals b. G+ pleomorph. chain cocci	75,000	a. S. fecalis b. G+ saprophytic cocci, rods and yeast cells	a. Possible disease of gall bladder b. Neg.

TABLE I—CONT'D

ACIDITY GASTRIC CONTENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION a. Unstained b. Stained (Gram)	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS a. Plate isolations b. Flask isolations	CLINICAL FINDINGS a. Clinical diagnosis b. Graham test
	NEG.	<100	<1000	>1000						
	8	4	1	6						
Hyperacidity: free, 35-80; total, 60-90			*Case* 112		3/ 6/29	F 29	a. Definite signs of stasis; microscop. findings favorable b. G+ pleomorph. chain cocci	250	a. Neg. b. S. albus	a. Possible disease gall bladder. Visceroptosis, migraine b. Negative
				Case 27	5/26/27	M 23	a. No "B" bile obtained "C" showed deeply bile-stained epithelia and pus b. No bacteria	5,000	a. A few contaminating cols. b. A few moulds	a. Possible disease gall bladder, migraine, spastic antrum on fluoroscopy b. Neg.
				61	3/ 2/28	F	a. Not suggestive of gall bladder disease b. No bacteria	250,000	a. B. coli communis, B. aerogenes b. B. coli communis, B. aerogenes	a. Cholecystitis (spasm and adhesions of antrum on fluoroscopy) b. Suggests pathology gall bladder
				85	10/10/28	M 54	a. Bile salts abund. Bile-stained mucus spirals and epith., cholest.-like crystals b. Large g+ rods	1,000,000	a. S. fecalis* b. S. fecalis*	a. Cholelithrasis (com. duct stone) b. No gall bladder shadow (2 tests)
				114	3/ 8/29	M 40	a. Definite evidence of marked stasis b. No bacteria	75,000	a. S. salivarius b. Saprophytic cocci and yeast	a. Hyperacidity, biliary migraine b. None made
				120	4/17/29	F 43	a. Definite evidence of stasis b. Large g+ cocci, g- bacilli	11,000	a. S. salivarius b. G+ polymorphic cocci	a. Hyperacidity, migraine b. None made

*No growth in 5 per cent bile, peptone broth.

TABLE I—CONT'D

ACIDITY GASTRIC CONTENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION a. Unstained b. Stained (Gram)	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS a. Plate isolations b. Flask isolations	CLINICAL FINDINGS a. Clinical diagnosis b. Graham test
	NEG.	<100	<1000	>1000						
	10	2	1	5						
Unclassified, no T. M.			*Case* 116		3/27/29	F 26	a. None made b. G+ med. diploc.	1,000	a. Nonhemol. polymorph. diplococci b. G+ nonhemolytic cocci	No record
				Case 11	3/15/27	F 46	a. None made b. G+ biscuit diplococcus; g+ rods	7,000	a. B. aerogenes b. B. aerogenes	a. Cholelithiasis b. Numerous gallstones
			VII	39	10/ 6/27	F 49	a. Cholesterin crystals, bile-stained epith. and pus b. No bacteria	None made	a. S. albus b. S. albus	a. Cholecystitis with stones
				39	10/ 6/27	F 49	a. "C" bile (immed. following "B") b. Occ. g+ med. chain coccus	None made	a. S. albus b. None seeded	b. Shows definite pathology gall bladder with many small stones
				63	3/14/28	F 52	a. No "B" portion, cholesterin crystals, lipoids and bile-stained mucus b. G+ sausage-shaped rods	650,000	a. B. acidi-lactici b. B. acidi-lactici	a. Cholelithiasis (attacks of colic) b. Suggests pathology gall bladder
				111	3/ 1/29	F 30	a. Clumped pigment, bile-stained epithelia and pus; mild infect. b. Occ. clump g+ pleomorph. cocci	500,000	a. Neg. b. Neg.	a. Chronic disease of gall bladder (jaundiced) b. Slightly suggestive of pathology gall bladder
				122	5/15/29	F 29	a. No "B" bile—3 attempts, patient very nervous b. Occasional large oval g+ coccus	13,000	a. S. equinus b. Large g+ coccus	a. Urticaria b. None made
	41	18	13	28						

bile. The balance of 21 cases, giving total counts ranging below one hundred, were considered of minor value in this analysis and are accordingly not being reported in such detail as are the other 37 positive cases. In each subgrouping where repeated examinations of bile from the same patient was made, the findings are brought together for convenience of comparison.

In Table II the same data are given for cases in which cultures were made from the duodenal content before washing out (D.B.W.) from the duodenum after washing out (D.A.W.) and from the "B" bile. The procedure in these cases was the same as already described except that after collection of the duodenal content, sterile water was introduced and withdrawn until it returned clear. Then the magnesium sulphate solution was introduced and the "B" bile specimen obtained in the usual way. The object of this experiment was to compare the flora of the duodenum both qualitatively and quantitatively with that of the "B" bile.

In the following paragraphs, the data recorded in Tables I and II are analyzed in an attempt to determine whether the culturing of "B" bile is a dependable guide to the nature of gall bladder infections, and if the bacterial types recovered may be accepted as suitable for the preparation of vaccines or if their source is so indefinite as to make their use unwarranted. The findings in our series of cases will be considered from the following standpoints:

1. Relation of cultural findings to clinical observations. Of the total 100 different cases there were 39 in the group yielding negative cultures and of these negative cases there were 28 or 70.2 per cent in which the clinical tests indicated cholecystitis. Of the 21 cases which showed fewer than 100 bacteria per c.c. in the bile there were 12 or 57.1 per cent with clinical evidence of cholecystitis. Of the 40 cases in the groups yielding more than 100 bacteria per c.c. of bile there were 31 or 77.5 per cent in which the clinical findings were positive for cholecystitis or at least suggested its possibility. There was accordingly no correlation between clinical evidence of gall bladder disease and quantitative bacterial findings; just as high a percentage showed sterile bile in cases clinically suggestive of gall bladder diseases as showed high bacterial counts. Of the 21 cases showing less than 100 bacteria per c.c. of bile there were 6 which yielded streptococcus and of these 4 resembled oral types which might have been brought down through swallowed saliva and 2 were Streptococcus fecalis; there were 2 biles yielding coliform organisms and 3 staphylococcus, 2 of them albus and 1 citreus. In the remaining nine cases in which organisms were recovered the types found were clearly saprophytic. Of the group of 40 cases yielding over 100 bacteria per c.c., there were 21 yielding streptococcus cultures of one type or another. Differential cultural tests (Holman's classification) indicated that 21 of the total 27 streptococcus strains isolated were of oral origin, viz., 7 salivarius, 7 mitis, 2 equinus, 1 subacidus and 3 not definitely identified. The remaining 6 conformed to intestinal types—Streptococcus fecalis (enterococcus). Only 5 of these strains were able to grow in the 5 per cent bile medium, viz., 3 Streptococcus fecalis and 2 Streptococcus salivarius. No frankly hemolytic types

TABLE II

BACTERIOLOGIC FINDINGS FOR THE DUODENAL CONTENT (BEFORE AND AFTER WASHING DUODENUM) AND THE "B" BILE

ACIDITY GASTRIC CONTENTS	CASE NO.	SOURCE OF MATERIAL	DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION	COLONY COUNT	BACTERIOLOGIC FINDINGS	CLINICAL FINDINGS
Hypo-acidity	134	D. B. W.	12/13/29	F 54	G+ diplococci, various sizes.	14,400	Staphylococcus albus (hemolyticus).	
		D. A. W.	12/13/29		G+ diplococci, yeast cells, few of each.	1,440	As above.	
		"B" bile	12/13/29		Dark viscid "B" fraction with few crystals, many pus cells and pigment in clumps. No bacteria noted.	20,000	As above.	Chronic cholecystitis; pathology of gall bladder; was to be operated.
Anacidity	123	D. B. W.	9/20/29	M 55	G+ cocci.		S. ignavus, B. coli communis, B. welchii.	
		D. A. W.	9/20/29		G+ cocci.		B. coli.	
		"B" bile	9/20/29		Stasis with stones, Giardia. G+ cocci, g– bacilli, spores estimated.	2,500,000	B. coli communior, about as many colonies as in D. A. W., B. welchii.	Chronic cholecystitis or pathology in liver; negative Graham test.
No T. M.	124	D. B. W.	9/20/29	F 22	G+ diplococci in long chains.	20,000	S. equinus, S. mitis, Streptococcus (nonhemolyticus).	
		D. A. W.	9/20/29		G+ cocci as in D. B. W. but scattered.	1,000	S. equinus.	
		"B" bile	9/20/29		"B" portion darker than normal; g+ diplococci, medium and large.	20,000	S. equinus, Strep. (nonhemolyticus).	Dermographism; improved from drainage. Negative Graham test.
No T. M.	128	D. B. W.	10/18/29	F 29	G+ cocci, pleomorphic.	150,000	Gram+ and g– cocci (like mouth saprophytes).	
		D. A. W.	10/18/29		No bacteria noted.	40,000	10 per cent as many colonies of same types as in D. B. W.	
		"B" bile	10/18/29		"B" portion normal except darker than usual. One clump g+ cocci.	2,500	Diphtheroid (sub-culture from flask).	Urticaria; definite improvement with drainage. Graham test not done.
No T. M.	136	D. B. W.	12/27/29	F 37	G+ cocci, medium and large, large g+ rod.	0	No growth.	
		D. A. W.	12/27/29		G+ cocci, few.	9,000	S. albus; S. (viridans).	
		"B" bile	12/27/29		Inky-black "B" portion; g+ cocci few.	250	S. albus, nonhemolyticus streptococcus from broth flask.	Angioneurotic edema; improved with drainage; Graham test not done.

were found. A few were alpha-prime and gamma as regards the changes on blood agar (Brown's classification) but most alpha (viridans). Fifteen cases yielded coliform bacilli and of these 12 were typical fermenters with gas in lactose and other differential carbohydrates while 3 did not split lactose or formed no gas. The B. aerogenes or the allied B. lactici-acidi were present in 10 cases and B. coli communis in only 3. A number of B. coli strains were tested for virulence by mouse inoculation but all yielded negative results. Where members of colon group were found at all, the count was high.

Of the cases with over 100 bacteria per c.c. in the bile, there were five which yielded staphylococci, in pure culture in 3, and mixed with streptococci in 2. All the former were the albus type.

Correlating the above bacterial findings with clinical diagnosis we find that of the 19 cases positive for one or another type of streptococcus, there were 13 for which the clinical history and findings indicated gall bladder disease although the Graham test was not positive for all. In 3 of these, however, the bile examined was apparently not the "B" fraction. On the other hand there were 6 cases all yielding "B" biles from which similar types of streptococci were isolated for which neither the clinical history nor findings indicated gall bladder disease. This would suggest that the streptococcal types found in drained bile are more likely to have had their origin in the intestine or the bile ducts rather than the gall bladder. This view is further substantiated by the fact that the streptococcal types found were mostly oral types, such as salivarius, which are seldom associated with pathologic processes, or fecal types (fecalis, enterococcus) of which the same may be said. The biles yielding B. coli were in a much higher percentage associated with gall bladder pathology than were those carrying streptococci. Eight of the 9 cases positive for B. coli or 88.8 per cent were clinically cholecystitis whereas only 46.1 per cent of the streptococcus-positive cases were so correlated. This might mean either that the finding of B. coli in "B" bile carries with it a greater assurance of gall bladder origin than does the streptococcus or that B. coli are more apt to be present in large numbers in the duodenum with gall bladder pathology, as has been claimed by Raue.[29] Repeated drainages, as will presently be indicated, favor the latter view. Staphylococcus albus is an organism of such feeble pathogenic propensities that we feel its presence in drained bile may well be considered of no significance and the same may be said of the great variety of other gram-positive coccal types which did not conform morphologically or culturally with the recognized pathogenic representatives of this group.

Finally the bile fractions submitted for culturing may be distributed among the following three groups: (a) satisfactory "B" portion; (b) doubtful "B" portion; (c) no "B" portion. This is based entirely on clinical observations at the time of drainage and, as is indicated in Table I, the number of satisfactory "B" portions was approximately double the number of the other two groups combined for the patients giving positive bacteriologic findings. The cultural results, however, showed no consistent and distinctive differences for these three classes.

2. RELATION OF THE CULTURAL FINDINGS TO GASTRIC ACIDITY

As has been stated the flora of the human duodenum is generally much richer, both as regards numbers and types, where gastric acidity is below normal or absent, than when conditions are normal in this respect. Accordingly if bacteria in the drained bile were derived in part or wholly from its passing through this region in the process of collection one might expect higher counts from cases of this type than from those in which gastric acidity is normal or above normal. Our findings as reported on this basis in Table I are to a certain extent consistent with this hypothesis. The combined figures for bile specimens from 35 cases with gastric anacidity or hypoacidity yielded biles in which the bacterial count per c.c. was above 100 numbered 17 or 48.6 per cent, whereas those from 44 cases with normal gastric acidity or hyperacidity gave counts per c.c. of bile above 100 in 34.1 per cent. In the former group streptococcal types of possible oral origin (salivarius, mitis, ignavus, etc.) were also somewhat more frequently encountered than in the latter: 11 times in 17 cases as contrasted with 6 times in 15 cases in the normal or above normal gastric acidity group. In the experience of one of us (J.C.T.) and that of others these are the types which are frequently found in material from the duodenum in connection with achylia gastrica.

3. CULTURAL FINDINGS IN CASES REPEATEDLY EXAMINED

There are six cases, recorded in Table I, in which more than one specimen of "B" bile was examined bacteriologically and at various time intervals. Logically, if the bacteria in the bile are those causing the cholecystitis one might expect that the cultural results would be consistent with a specific infection. This, however, was by no means always the case. In group I, for instance, the first drainage yielded a pure culture of B. coli communis but the second taken fifty-two days later a pure growth of B. lactis aerogenes. Group II in the first drainage showed Streptococcus fecalis, but in the second, performed nine days later, only Streptococcus mitis was isolated from both the plates and the culture flask. Group III consistently on both occasions yielded B. lactici acidi. Group V in drainages performed four months apart yielded entirely different types of streptococci and group VI different types of B. coli. Bartle[31] explains such diversity in findings from repeated examinations of a single case as due to a multiple infection of the gall bladder, but it would seem quite as logical to ascribe them to changes in the bacterial types predominant from time to time in the duodenum and jejunum and hence certain bacterial types might be picked up during the drainage process at one time and others at another. These repeated drainages yielded one quite consistent finding and that of a quantitative nature: if high bacterial counts were obtained in one examination the same was likely to occur at subsequent examinations.

4. CULTURAL FINDINGS FOR THE DUODENUM AND THE HOMOLOGOUS "B" BILE

The data assembled in Table II demonstrates clearly that one is not justified in assuming that the bacterial types isolated from the "B" bile were derived from the gall bladder. In these five cases, as stated in a preceding

paragraph, samples from the duodenum before and after washing that region were obtained and then one of the "B" bile. Two of the patients showed subnormal gastric acidity, while for the other three no gastric analyses were made. In Case 134 a Staphylococcus albus in high count was the only finding for all three specimens. In Case 123, B. coli and B. welchii were recovered from the duodenal content and the "B" bile. The only difference in the findings lay in the fact that the B. coli of the duodenum conformed culturally to communis whereas that of the bile to communior. It seems probable that if a considerable number of colon colonies had been fished and identified that both of these types might have been found in each specimen. In Case 124 we have almost exactly the same findings for the duodenal content and the "B" bile; Streptococcus equinus and another nonhemolytic streptococcus of identical type in each. Case 128 yielded gram-positive and gram-negative unidentified cocci for both the specimens of duodenal content. In the "B" bile these were not found, but from it a diphtheroid-like organism was isolated. Case 136 yielded no growth from the duodenal content before washing, a Staphylococcus albus and a viridans streptococcus of indefinite nature from the "B" bile. In three of these cases the findings in the "B" bile were thus practically the same as for the duodenal content and in the other two the organisms recovered were strongly suggestive of mouth origin in all three specimens. In no instance was any organism of probable pathogenic nature isolated from the "B" bile which had not also been found in the "before washing" or "after washing" duodenal specimens. The colony counts also indicate that even after the duodenum has been washed with sterile water until the fluid is returned clear there are enough bacteria left in the region of the bile duct opening to account in many instances for the numbers and types of bacteria found in the "B" bile. In two of the patients, Cases 128 and 134, the types recovered suggest derivation from swallowed saliva while in the other three the organisms constituted the flora of the duodenal drainage field with possible accessions from lower levels through reversed peristalsis incident to the irritation caused by the presence of the duodenal tube tip.

5. COMPARISON OF "B" BILE BACTERIAL FINDINGS WITH REPORTS FOR INFECTED GALL BLADDERS

In Table III we have assembled from recent literature data in regard to the bacterial content of the gall bladder as determined for operated cases. Our immediate purpose in tabulating these findings was to compare them as regards types of bacteria found and their respective percentages of incidence with those which are reported for "B" bile in this article and also by Lyon and Kolmer[8] and by Bartle.[31] First as regards sterility of the bile in patients operated upon for gall bladder disease, both acute and chronic, we find that of the total 780 examinations, including all reports in which 50 or more cases were investigated, there was an average of 66 per cent sterile, with a range of 45 per cent as reported by Rosenow to 88 per cent as found by Wilkie. In the Lyon and Kolmer series the drained "B" bile was found sterile in 45 per cent of instances, in a series of 200 consecutive cases reported on by Bartle, there were only 10 per cent sterile "B" biles and in our series 39.3 per cent. In

TABLE III

SUMMARY OF BACTERIOLOGIC FINDINGS FOR GALL BLADDERS AT OPERATION AS REPORTED IN THE LITERATURE

AUTHOR	CASES		SOURCE OF SPECIMEN	BACTERIAL TYPES ISOLATED (PER CENT)										
	ACUTE	CHRONIC		COLIFORM	B. TYPHOSUS	STAPHYLOCOCCUS	STREPTOCOCCUS	ENTEROCOCCUS	B. MUCOSUS	DIPHTHEROID	B. WELCHII	UNIDENTIFIED	STERILE	
Kelly	240		Bile	29.2	11.3	4.6	0.5 Pyogenes	–	–	–	–	2.5	53.0	240 presumably routine cases
Rosenow			Wall 61 cases	36.1	–	3.3	60.7	–	8.2	8.2	3.3	–	16.4	
			Bile 55 cases	32.7	–	9.1	23.6	–	5.6	1.8	–	–	45.5	
	14	61	Stone 63 cases	17.5	3.2	–	77.8	–	–	–	16.0	–	9.5	
Brown	20	50	Wall	17.0	–	–	43.0	–	–	–	–	–	49.0	70 unselected cases
Whipple	3	22	Wall	16.0	–	12.0	20.0	8.0	–	–	–	–	4.0	25 operative cases
			Bile	32.0	–	8.0	20.0	4.0	–	–	–	–	32.0	
Drennan	100 Mostly chronic		Bile 19+	12.0	–	4.0	2.0 Hemolyticus	–	–	–	–	–	81.0	100 consecutive operative cases
Johnson	12	86	Wall	18.0	1.0	12.0	3.0	–	–	–	–	–	68.0	Histologic diagnosis on 100 consecutive operative cases
			Bile 32+ Stone 50 cases	20.0	1.0	8.0	11.0	–	–	–	–	–	–	
Judd, Mentzer and Parkhill	No cases operated in acute stage		Wall 200 cultured 98+	9.5	–	10.0	14.0	–	–	0.5	1.0	6.5	51.0	200 operative cases Contamination in 25 cases
			Bile 193 cultured 28+	6.2	–	1.1	7.8	–	–	–	0.5	3.6	85.5	Gallstones in 107 cases
			Stone 67 cultured	3.0	–	–	9.0	–	–	–	–	–	64.2	

TABLE III—CONT'D

AUTHOR	CASES		SOURCE OF SPECIMEN	BACTERIAL TYPES ISOLATED (PER CENT)										
	ACUTE	CHRONIC		COLIFORM	B. TYPHOSUS	STAPHYLOCOCCUS	STREPTOCOCCUS	ENTEROCOCCUS	B. MUCOSUS	DIPHTHEROID	B. WELCHII	UNIDENTIFIED	STERILE	
Illingsworth	12	88	Wall 100 cultured 62+	22.0	–	6.0	39.0	–	–	–	–	0	38.0	100 presumably routine cases
			Bile 100 cultured 40+	21.0	–	3.0	17.0	–	–	–	–	0	60.0	
			Stone 23 cultured 7+	13.0	–	8.7	8.7	–	–	–	–	0	69.5	
			Cystic gland 15 cultured 6+	13.3	–	0	20.0	–	–	–	–	6.7	60.0	
Wilkie	20	30	Wall all coats	6.0	–	–	4.0	–	–	–	2.0	–	88.0	50 operative cases
			Bile	6.0	–	–	4.0	–	–	–	2.0	–	88.0	
			Sub. mucosa	–	–	–	42.0	–	–	–	2.0	–	56.0	
			Cystic gland	2.0	–	–	86.0	–	–	–	2.0	–	10.0	
Branch	57	153	Wall 210 cultured 53+	12.3	0.5	4.3	2.9	–	–	–	1.9	1.0	74.8	210 routine operative cases
			Bile 210 cultured 40+	9.5	0.5	3.3	2.8	–	–	–	1.0	0.5	81.0	Percentages recalculated to total cases examined, as reported in Branch's Table II
			Stone 94 cultured 27+	14.9	2.0	5.3	2.1	–	–	–	2.1	1.0	71.3	
Nickel and Judd	96	82	Wall	15.0	–	13.0	22.0	–	–	–	–	–	50.0	300 consecutive operative cases
			Cystic gland	Cultures from cystic lymph nodes approximately parallel to those from wall										

this total of 364 cases in which the drained bile was examined bacteriologically there was thus an average of approximately 33 per cent which were found sterile or one-half the average reported for gall bladder bile. This would indicate, then, that, assuming all the "B" biles were derived from the gall bladder, the bacteria in at least one-third were added to the bile during the process of drainage either from above or below the drainage area in the intestine or from the hepatic ducts. This table also brings out the fact that without exception where both the gall bladder wall and bile were examined bacteriologically, the former yielded the higher percentage of positive cultures with an excess of positives for wall over bile ranging from 24 (Branch) to 73 (Wilkie, submucosa) per cent. Illingsworth[32] in commenting on his cultural findings makes the following statement which is applicable to a greater or less degree to most of the other reports included in the tables. "In relation to diagnosis the investigation here recorded is of interest as it affects the Meltzer-Lyon test, for, in view of the occurrence of uninfected bile in 60 per cent cases it seems obvious that a negative bacteriologic finding in this examination must be of no significance and in fact it does not even exclude the presence of gross gall bladder disease." To which on the basis of our findings for drained "B" bile we may add the conclusion that positive bacterial findings for this material in no way justifies the assumption that the organisms recovered came from the gall bladder. Wilkie[33] found further that the streptococci recovered from the submucosa of the gall bladder were inhibited and ultimately killed by the bile taken from the same gall bladder. Hence, he concludes, it is unreasonable to expect to find the infecting streptococcus in drained bile. He found also that a far higher percentage of streptococci could be recovered from the cystic lymph gland which drains the entire submucous and outer coats of the gall bladder than from the bile; his cases yielding 86 per cent positive findings for the former as against 4 per cent for the latter.

This Table III also reveals that 7 out of 8 of those who made qualitative bacterial studies of the gall bladder bile found B. coli more frequently than streptococci, but the reverse was true for the gall bladder wall. In our series of cases, however, streptococci were more frequently recovered than B. coli from the "B" bile with the respective percentages of positive findings of 44.2 and 28. These differences in prevalence of types in gall bladder bile and drained "B" bile further add to the uncertainty as to the origin of the organisms found in the latter.

CONCLUSIONS

1. A report is given on the bacteriologic findings for bile recovered by the Lyon Technic in 100 consecutive cases. The following conclusions are drawn:

We are unable to establish any satisfactory correspondence between bacterial findings for the drained bile and clinical tests for cholecystitis. Just as high a percentage of cases clinically suggestive of gall bladder disease yielded sterile biles as high bacterial counts.

Of the various types of bacteria recovered B. coli was found to be much more frequently associated with positive tests for gall bladder pathology than

were streptococci. It seemed probable that the latter, at least, were frequently present as contamination of the drained bile through contact with swallowed saliva or from the flora of the duodenum. This conclusion was substantiated by the fact that frequently the types recovered could not thrive in 5 per cent bile, peptone broth.

In 6 cases in which more than one specimen of drained bile was examined at various time intervals there were 4 in which the findings were not consistent for an assumed specific infection of the gall bladder. This suggests an extrahepatic origin of the bacteria.

In 5 cases in which the duodenal flora was determined before and after washing with sterile water and then compared with that of the "B" bile it was found that there was sufficient resemblance to warrant the conclusion that the bile might have derived its organisms either from the duodenal flora or from swallowed saliva. This applies not only to streptococci and staphylococci but also to B. coli.

The above facts and also a comparison of our bacteriologic findings and those of others for "B" biles with similar reports in the literature on direct culture of gall bladder bile at operation show certain inconsistencies which justify great hesitancy in making use of bacterial cultures isolated from drained bile in the preparation of autogenous vaccines.

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A SURVEY OF INTESTINAL PROTOZOA AMONG CHILDREN IN ST. LOUIS*

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THIS survey was undertaken with the view of determining primarily incidence of intestinal protozoa among 362 white children in St. Louis as compared with that found elsewhere, and second to ascertain if there existed differences in the incidence under varying conditions with respect to the general state of health and environment of children. The subjects were, therefore, taken from three distinct groups. The first group consisted of 164 inmates of two orphanages (110 in one orphanage and 54 in the other). A great majority of these children were apparently healthy individuals. Hygienic conditions in the first orphanage were, however, much inferior to those in the second where particular emphasis is laid on sanitation and personal habits. The second group was represented by 156 patients of two hospitals for convalescents (51 in one hospital and 105 in the other). These children were still under periodic supervision of attending physicians, and most of them were suffering from various chronic ailments. Conditions with respect to hygiene and sanitation were up to standard in both hospitals. The third group consisted of 42 hospital bed-patients suffering from acute illnesses, and these children were under constant care of physicians and nurses.

In many instances positive findings were secured on the first examination. When the findings were negative, however, the second specimen was secured but in no case was the examination made more than twice. Each sample of stool was examined several times and if cysts were not found by ordinary method Riva's modification[1] of the ether-acetic acid concentration method was used to verify negative finding. Direct smears were prepared from each sample of stool and emulsified in normal saline, Gram-iodin and 1-10000 aqueous eosin solutions respectively. The culture media such as Hogue's ovomucoid medium[2] and Boeck and Drbohlav's medium[3] were used whenever necessary. Permanent slides were prepared of all positives by the Heidenhain iron-hematoxylin method and were utilized for a final diagnosis whenever it was difficult to determine with certainty the species present in direct smears.

As shown in Table I, the incidence of intestinal protozoa among children as influenced by the general state of health seemed to show no significant differences among the three groups. If the general state of health were a determining factor, lower incidence should have been found in the first group (healthy children) than in the other groups which included children whose vitality was low. We found, however, that the healthy children in one of the orphanages (A) showed the highest percentage of infection, while the lowest

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TABLE I

A COMPOSITE TABLE SHOWING THE INCIDENCE OF PROTOZOAN INFECTIONS OF INTESTINAL TRACT AMONG CHILDREN IN ST. LOUIS

GROUP	I (164 INMATES)				II (156 PATIENTS)				III (42 PATIENTS)		TOTAL (362)	
INSTITUTION	A. (110)		B. (54)		C. (51)		D. (105)					
PROTOZOAN SPECIES	BOY (78)	GIRL (32)	BOY (31)	GIRL (23)	BOY (28)	GIRL (23)	BOY (49)	GIRL (56)	BOY (27)	GIRL (15)	BOY (213)	GIRL (149)
Endameba coli	12	6	3	2	4	–	7	2	2	1	28	11
Endameba histolytica	3	–	–	–	–	–	–	–	–	–	3	0
Endolimax nana	–	1	–	–	1	–	2	3	–	–	3	4
Iodameba williamsi	–	1	1	–	2	–	–	–	–	–	3	1
Chilomastix mesnili	–	1	–	–	–	–	–	2	1	–	1	3
Giardia lamblia	17	8	6	2	6	6	7	8	3	–	39	24
Trichomonas hominis	–	–	–	–	–	–	–	–	–	–	0	0
Total	32	17	10	4	13	6	16	15	6	1	77	43
Double Infections												
G. lamblia and E. coli	10	3	–	–	1	2	–	2	1	–	12	7
G. lamblia and C. mesnili	1	1	–	–	–	–	–	–	–	–	1	1
G. lamblia and E. histolytica	1	–	–	–	–	–	1	–	–	–	2	0
G. lamblia and I. williamsi	1	–	–	–	–	–	–	–	–	–	1	0
G. lamblia and E. nana	–	–	–	–	–	–	1	–	–	–	1	0
E. coli and E. nana	1	–	–	–	–	1	–	–	–	–	1	1
E. coli and C. mesnili	1	–	–	–	1	–	1	1	1	–	4	1
E. coli and I. williamsi	1	1	–	–	–	–	–	–	–	–	1	1
E. coli and E. histolytica	1	–	–	–	–	–	–	–	–	–	1	0
E. histolytica and E. nana	–	–	–	–	–	–	1	–	–	–	1	0
E. nana and I. williamsi	–	1	–	–	–	–	–	–	–	–	0	1
Total	17	6	0	0	2	3	4	3	2	0	25	12
Triple Infections												
G. lamblia, E. coli and E. nana	–	–	–	–	1	–	–	1	–	–	1	1
G. lamblia, E. coli and T. hominis	–	–	–	–	1	–	–	–	–	–	1	0
G. lamblia, E. coli and C. mesnili	2	1	–	–	–	–	1	–	–	–	3	1
G. lamblia, E. coli and E. histolytica	1	–	–	–	–	–	–	–	–	–	1	0
G. lamblia, E. coli and I. williamsi	1	–	–	–	–	–	–	–	–	–	1	0
G. lamblia, C. mesnili and I. williamsi	1	–	–	–	–	–	–	–	–	–	1	0
G. lamblia, C. mesnili and E. histolytica	1	–	–	–	–	–	–	–	–	–	1	0
Total	6	1	0	0	2	0	1	1	0	0	9	2
Grand total positive	55	24	10	4	17	9	21	19	8	1	111	57
Grand total negative	23	8	21	19	11	14	28	37	19	14	102	92

incidence occurred among sick children in the third group. Furthermore, there was no mixed infection found in the latter group while in the former the incidence of such was surprisingly high, and in seven out of nine instances E. histolytica was found. On the other hand, the second orphanage (B) where a rigid hygiene program is maintained showed a comparatively low incidence.

Of the total of 362 children examined, in 120 (33.09 per cent) the infection was limited to one protozoan species; in 37 (10.22 per cent) a double infection was found, while in 11 children (3.07 per cent) a triple infection was noted. Among the intestinal protozoa, the incidence of Giardia lamblia was the highest, with the following species appearing in order of frequency, Endameba coli, Chilomastix mesnili, Endolimax nana, Endameba histolytica, Iodameba williamsi and Trichomonas hominis. In the majority of mixed infections Giardia lamblia was associated with either one or two other protozoa, of which Endameba coli was found most commonly.

The present survey revealed one of the highest incidences of intestinal protozoan infections among children reported in this country. This may be accounted for by the geographic location of St. Louis. In this study the incidence of Giardia lamblia (27.3 per cent) was lower than that reported by Boeck[4] in a survey of an industrial school for boys and girls (49.3 per cent), and that reported by Hill and Hill[5] among children in Porto Rico (47.2 per cent), and was considerably higher than those of Maxcy[6] (15.7 per cent), Tansinsin[7] (13 per cent), and others. If in this study the number of examinations per child had approached that of Boeck (5.3 examinations per child) instead of being limited to two examinations, the incidence probably would have been found even higher. The incidence of Endameba coli was significantly greater in this survey than in most of the others reported. Whether G. lamblia and E. coli can establish themselves in the intestine of children more readily than other protozoa or whether their wider natural distributions in this locality are responsible for the greater incidence is difficult to determine from this study. It may be suggested, however, that children are constantly exposed not only to the possibility of single or mixed infection, but also to auto-reinfection by same protozoan species. The incidence of Endameba histolytica in this series was not high as compared with some of the surveys made elsewhere. The scarcity of Trichomonas hominis in this study seemed

TABLE II

A COMPOSITE TABLE SHOWING AGE DISTRIBUTIONS OF PROTOZOAN INFECTIONS OF INTESTINAL TRACT AMONG CHILDREN IN ST. LOUIS

AGE	PROTOZOA ENDAMEBA HISTOLYTICA	ENDAMEBA COLI	ENDOLIMAX NANA	IODAMEBA WILLIAMSI	CHILOMASTIX MESNILI	GIARDIA LAMBLIA	TRICHOMONAS HOMINIS	DOUBLE INFECTION	TRIPLE INFECTION	POSITIVE TOTAL	NEGATIVE TOTAL
1–3	–	1	–	–	–	7	–	1	–	9	34
4–6	1	8	2	3	–	9	–	11	4	38	34
7–9	1	14	4	–	–	25	–	16	4	64	53
10–12	1	11	1	1	2	19	–	6	2	43	39
13–16	–	5	–	–	2	3	–	3	1	14	34
Total	3	39	7	4	4	63	0	37	11	168	194

to be due to coincidence rather than to the types of samples received or the method employed for its detection. As a matter of fact, a great majority of these specimens were examined within a few hours after collection and some of them immediately. Moreover, Hegner[8] claimed that Trichomonas hominis, when kept at room temperature, exhibited no apparent diminution in number for the first few hours.

The ages of children in this survey ranged from one to sixteen years, and were tentatively placed into five groups: one to three, four to six, seven to nine, ten to twelve and thirteen to sixteen years old. A relative frequency of the intestinal protozoa based on age distribution is shown in Table II.

The highest incidence of intestinal protozoa was found among children of age-group seven to nine years with the following groups appearing in order of their frequency, ten to twelve, four to six, thirteen to sixteen and one to three years old. In all instances, the incidence was higher among boys (111 positive out of 213) than among girls (57 positive out of 149).

Faust[9] believes that except for infections with Giardia there is a definite increase in the protozoan incidence from childhood to middle age. The present report shows a sudden fall in the incidence of all the protozoa as children became older. Thus, an apparent fall was observed in the group of children from thirteen to sixteen years old. A greater incidence among children between four and twelve years old may be explained by the fact that these childrn come in close personal contact with their playmates, some of whom are undoubtedly carriers of intestinal protozoa. Irrespective of the general state of health of individuals, they may become carriers, if sufficiently exposed to an infection by ingestion of cysts or trophozoites with food or drink.

As bearing on the question of pathogenicity of these protozoa an attempt was made to ascertain the prevalence of constipation or diarrhea among the infected children. Despite the fact that diarrhea has been considered by many as a cardinal symptom of intestinal protozoan infections, no correlation seemed to exist between intestinal protozoa and diarrhea in this study. Thus, even among children who harbored abundant cysts of Endameba histolytica

TABLE III

THE INCIDENCE OF INTESTINAL PROTOZOA AS CORRELATED WITH DAILY HABITS OF 105 CHILDREN IN GROUP II D (CONVALESCENTS)

BOWEL MOVEMENT	PROTOZOA ENDAMEBA COLI	ENDOLIMAX NANA	GIARDIA LAMBLIA	CHILOMASTIX MESNILI	ENDAMEBA HISTOLYTICA GIARDIA LAMBLIA	ENDAMEBA COLI CHILOMASTIX MESNILI	GIARDIA LAMBLIA ENDOLIMAX NANA	GIARDIA LAMBLIA ENDAMEBA COLI	ENDAMEBA HISTOLYTICA ENDOLIMAX NANA	ENDAMEBA COLI GIARDIA LAMBLIA ENDOLIMAX NANA	ENDAMEBA COLI GIARDIA LAMBLIA CHILOMASTIX MESNILI	NEGATIVE	TOTAL
Normal	8	4	11	1	1	1	–	1	1	1	1	52	82
Constipation	1	1	4	1	–	1	1	1	–	–	–	12	22
Diarrhea	–	–	–	–	–	–	–	–	–	–	–	1	1
Total	9	5	15	2	1	2	1	2	1	1	1	65	105

there was no history of diarrhea, neither was there any diarrhea accompanying the presence of Giardia lamblia. This may be explained by assuming that a great majority of these children were "carriers," since in none of them was there the symptom that has been referred to by many as characterizing the protozoan infections of intestinal tract.

Of the total 105 children examined, 65 were negative for protozoa, 52 had a record of normal bowel movements, 12 were constipated, and 1 had diarrhea. Among 21 children harboring G. lamblia, there were 15 had a record of normal movements and 6 tended to be constipated and no cases of diarrhea. In two instances of E. histolytica associated with other protozoa the movements were reported to be regular. Moreover, the presence of a large number of protozoan cysts in one examination of stool does not seem to indicate the severity of infection, as there is constant fluctuation in the number of discharged cysts of intestinal protozoa from day to day as has been recently reported by me[10] on Giardia lamblia.

SUMMARY

1. A survey of intestinal protozoa was conducted among three distinct groups of white children in St. Louis under varying conditions with respect to their general state of health and sanitary environment. Of 362 children examined, there were 164 healthy inmates of two orphanages, 156 patients from two hospitals for convalescents and 42 patients from a hospital for acute illnesses.

2. Among the healthy children in one of the orphanages (A) where personal hygiene was not sufficiently emphasized, the incidence was much higher than among sick children temporarily confined in the hospital. On the other hand, in the other orphanage (B) where a rigid hygiene was enforced, there was a very low incidence. This indicates that, irrespective of the general state of health of children, sanitary and hygienic conditions play an important rôle in the transmission of the protozoan infections.

3. The age-groups showed that among children between seven and nine years, the incidence was the highest while the lowest was between one and three years. A very low incidence was observed among children between thirteen and sixteen years old indicating a fall in positive instances as children become older. The incidence was higher among boys than among girls.

4. There was no correlation between the presence of intestinal protozoa and diarrhea. As a matter of fact, there was no single case of diarrhea among those positive (40) for protozoa, with one among those negative (65). This may be accounted for by the fact that a great majority of these children were carriers of various intestinal protozoa.

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BIOCHEMICAL AND PHARMACOLOGIC STUDY OF QUININE BI-SALICYLO-SALICYLATE*

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INTRODUCTION

THE salicylates and quinine compounds are among the most extensively investigated drugs, both experimentally and clinically. During the past half century or more many compounds of salicylic acid and of quinine have been introduced to the medical public, but none is of more peculiar interest than this recently produced compound of quinine and salysal (quinine bi-salicylo-salicylate). The nearest approach to this compound that had formerly been made is quinine salicylate, yet, the two compounds are quite different in their chemical properties and in their physiologic reactions. Perhaps, the most important difference between the two compounds is their reaction in acid media such as gastric juice. Quinine salicylate hydrolyzes in the stomach into quinine and salicylic acid, and, therefore, on digestion exhibits all the untoward reactions of free salicylic acid. Quinine bi-salicylo-salicylate hydrolyzes in the stomach into quinine and salicylo-salicylate (salysal). Now this salicylate compound, which may be considered a salicyl ester of salicylic acid, is practically insoluble in acid media such as gastric juice and hence does not liberate free salicylic acid in the stomach. It is therefore very much less irritating during the period just following ingestion than quinine salicylate. Hanzlick and Prasho† showed that salicylo-salicylate (salysal) passes through the stomach unchanged, and that it is only after it reaches the alkaline medium of the intestines that salicylic acid is liberated, and after that absorption slowly takes place. In other words, the alkali of the intestines hydrolyzes the salicylo-salicylate into free salicylic and then converts it into sodium salicylate, which is soluble.

The chemical characteristics of quinine bi-salicylo-salicylate may be briefly listed as follows: It is a white, odorless, crystalline substance having a slightly bitter taste. It is practically insoluble in water, but in acid media such as gastric juice it separates into quinine and salicylo-salicylate. The quinine content, expressed as quinine U.S.P., $3H_2O$, is 45.1 per cent (quinine salicylate contains 78.9 per cent). The salicylic acid content is 65.8 per cent (quinine salicylate contains 28.7 per cent).

The physiologic actions of quinine and salicylate acid are known to overlap in many respects, but it is evident that their similar effects are produced

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in different manners. Quinine acts as a stimulant to the nervous system; it is a slight respiratory stimulant and a depressant to the circulation; it lessens the ameboid movement of the white corpuscles; it possesses a mild antiseptic action; and it reduces fever by diminishing heat production by a peripheral depression of nitrogenous metabolism. In other words, quinine affects the general metabolism of all forms of protoplasm, and in large doses acts as a general protoplasmic poison. Salicylic acid, on the other hand, is stated to exert its beneficial therapeutic influence through its specific antiseptic effect and it reduces fever by increasing heat dissipation.

From the above remarks, it is evident that this new compound, quinine bi-salicylo-salicylate, should be useful whenever combined quinine and salicylic medication is indicated, particularly in painful and febrile diseases, such as rheumatism, tonsillitis, influenza, and neuralgia.

The object of the present study was to determine the biochemical properties and pharmacologic action of this compound as compared with analogous experimental quantities or doses of quinine in the forms of quinine sulphate and quinine salicylate, and salicyl in the forms of sodium salicylate, salicylo-salicylate, and quinine salicylate, etc.

SOME CHEMICAL REACTIONS OF QUININE BI-SALICYLO-SALICYLATE

1. Aqueous suspension of quinine bi-salicylo-salicylate + $FeCl_3$ solution (salicylic acid test) = light purple. The color intensified on standing.

2. Aqueous suspension of quinine bi-salicylo-salicylate + dil. HCl solution and heat; then neutralized and treated with $FeCl_3$ solution = purple color. The reaction, however, was only slightly more positive than that obtained in Exp. 1, thereby showing that dilute hydrochloric acid is not a good hydrolizing agent for quinine bi-salicylo-salicylate.

3. Aqueous suspension of quinine bi-salicylo-salicylate + NaOH solution, plus heat; then neutralized and treated with $FeCl_3$ solution = intense purple color. It is evident that hydrolysis was good and that sodium salicylate was formed. Of course the neutralizing of the mixture liberated free salicylic acid.

4. Aqueous suspension of quinine bi-salicylo-salicylate plus very dilute H_2SO_4 solution, plus heat, plus alkaloidal reagents yielded the usual alkaloidal precipitation.

5. Aqueous suspension of quinine bi-salicylo-salicylate on filtering and treating the filtrate with alkaloidal reagents gave the usual precipitate.

6. Some of the filtrate from Exp. 5 on treating with dilute H_2SO_4 became fluorescent (quinine reaction).

7. Quinine salicylate plus water, plus $FeCl_3$ solution gave a dark purple color immediately.

8. Acetylsalicylate plus water, plus $FeCl_3$ solution gave a dark purple color immediately.

9. Quinine bi-salicylo-salicylate on treatment with ether leaves a residue which gives a dark purple color on treatment with $FeCl_3$ solution.

10. Quinine bi-salicylo-salicylate is readily soluble in cold chloroform and in hot acidified alcohol.

11. In aqueous solutions of varying P_H it was found that quinine bi-salicylo-salicylate is most soluble in alkaline solutions of about P_H 10, next in acid solutions of about P_H 2 and least soluble in solutions of P_H 7.

DIFFUSION OF QUININE BI-SALICYLO-SALICYLATE THROUGH COLLODION SACS AT DIFFERENT P_H

Each sac contained 1 gram of quinine bi-salicylo-salicylate plus 20 c.c. of water of the varying P_H indicated below, and was allowed to diffuse into water of the same P_H.

TABLE I

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min.	$FeCl_3$ test	−	−	−	−	−	−	−
	Alk. ppt.	−	−	−	−	−	−	−
30 min.	$FeCl_3$ test	−	−	−	−	−	−	−
	Alk. ppt.	−	−	−	−	−	−	−
1 hr.	$FeCl_3$ test	−	−	−	−	−	−	−
	Alk. ppt.	−	−	−	−	−	−	−
2 hr.	$FeCl_3$ test	+−	+−	+−	−	−	−	+
	Alk. ppt.	−	−	−	−	−	−	−
3 hr.	$FeCl_3$ test	+−	+	+−	+−	−	+−	+++
	Alk. ppt.	−	−	−	−	−	−	−
6 hr.	$FeCl_3$ test	+	++	+	+	+	++	++++
	Alk. ppt.	+	+	+	+	+	+	++
24 hr.	$FeCl_3$ test	++	+++	++	+	+	++	++++
	Alk. ppt.	++	+++	++	++	+	+	+++

It will be noted that diffusion was greatest at P_H 10 and next at P_H 2.

DIFFUSION OF QUININE BI-SALICYLO-SALICYLATE IN THE PRESENCE OF PROTEIN SOLUTION AT VARYING P_H

Each diffusion sac contained 1 gram of quinine bi-salicylo-salicylate suspended in 20 c.c. of water of different P_H plus 5 c.c. of a saturated solution of egg albumen. The diffusion was into solutions of the same P_H.

TABLE II

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min.	$FeCl_3$ test	−	−	−	−	−	−	−
	Alk. ppt.	−	−	−	−	−	−	−
30 min.	$FeCl_3$ test	−	−	−	−	−	−	−
	Alk. ppt.	−	−	−	−	−	−	−
1 hr.	$FeCl_3$ test	−	−	−	−	−	−	+
	Alk. ppt.	−	−	−	−	−	−	−
2 hr.	$FeCl_3$ test	−	−	−	−	−	−	+
	Alk. ppt.	−	−	−	−	−	−	−
3 hr.	$FeCl_3$ test	+−	+	+−	−	−	+−	++
	Alk. ppt.	−	−	−	−	−	−	−
6 hr.	$FeCl_3$ test	+	++	+	+−	+	+−	++
	Alk. ppt.	+	+	+	+	+	+	+
24 hr.	$FeCl_3$ test	++	++	+	+	++	+	+++
	Alk. ppt.	+	++	+	+	+	+	+
48 hr.	$FeCl_3$ test	+++	+++	++	+	++	++	++++
	Alk. ppt.	+++	++++	++	++	++	++	++++

These results indicate that albumin does not interfere with the hydrolysis and diffusion of quinine bi-salicylo-salicylate, i.e., the results are practically the same as those obtained in the previous table.

DIALYSIS OF QUININE SULPHATE PLUS SODIUM SALICYLATE FROM SOLUTIONS OF DIFFERENT P_H

Each diffusion sac contained ½ gram of quinine sulphate plus ½ gram of sodium salicylate dissolved in 20 c.c. of water of different P_H and were diffused into solutions of the same P_H.

TABLE III

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min.	$FeCl_3$ test	−	+−	+	+	+++	+	−
	Alk. ppt.	−	−	−	−	−	−	−
30 min.	$FeCl_3$ test	−	+++	+++	++	++++	++	+
	Alk. ppt.	−	−	−	−	−	−	−
1 hr.	$FeCl_3$ test	+−	++++	++++	+++	++++	+++	+
	Alk. ppt.	−	−	−	−	−	−	−
2 hr.	$FeCl_3$ test	+	++++	++++	++++	++++	++++	++++
	Alk. ppt.	+	+	+	+	−	−	−
3 hr.	$FeCl_3$ test	++++	++++	++++	++++	++++	++++	++++
	Alk. ppt.	+++	++	+	+	−	−	−
6 hr.	$FeCl_3$ test	++++	++++	++++	++++	++++	++++	++++
	Alk. ppt.	++++	++++	++++	++	−	−	−
24 hr.	$FeCl_3$ test	++++	++++	++++	++++	++++	++++	++++
	Alk. ppt.	++++	++++	++++	++	−	−	−
48 hr.	$FeCl_3$ test	++++	++++	++++	++++	++++	++++	++++
	Alk. ppt.	++++	++++	++++	+++	+−	−	−

Table III presents quite a different picture from the results obtained with quinine bi-salicylo-salicylate. In this case P_H 7 is the reaction point at which the salicylate is most diffusible and the maximum rate is attained within thirty minutes. The quinine is diffusible in acid medium only and starts between one and two hours; the maximum rate being attained at about six hours.

DIALYSIS OF QUININE SULPHATE PLUS SODIUM SALICYLATE IN THE PRESENCE OF PROTEIN SOLUTIONS OF VARYING P_H

In each diffusion sac were placed ½ gram of quinine sulphate, ½ gram of sodium salicylate, 20 c.c. of water of a given P_H and 5 c.c. of saturated egg albumen solution. These were placed in water of the same P_H as that present in each sac.

TABLE IV

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min.	$FeCl_3$ test	−	−	−	−	−	−	−
	Alk. ppt.	−	−	−	−	−	−	−
30 min.	$FeCl_3$ test	−	+	+−	+−	++	+−	−
	Alk. ppt.	−	−	−	−	−	−	−
1 hr.	$FeCl_3$ test	+−	++	+	+	++++	+	−
	Alk. ppt.	−	−	−	−	−	−	−
2 hr.	$FeCl_3$ test	+	+++	++	+	++++	++	+−
	Alk. ppt.	+	+	+	−	−	−	−
3 hr.	$FeCl_3$ test	+	++++	+++	+	++++	+++	+
	Alk. ppt.	++	++	++	−	−	−	−
6 hr.	$FeCl_3$ test	++	++++	++++	++	+++	++++	++
	Alk. ppt.	++++	++++	++++	+−	−	−	−
24 hr.	$FeCl_3$ test	++++	++++	++++	++++	++++	++++	++++
	Alk. ppt.	++++	++++	++++	+	+	+	−

The results of this experiment apparently indicate that albumin has a greater retarding effect on the diffusion of quinine sulphate and sodium salicylate than it does on quinine bi-salicylo-salicylate.

DIALYSIS OF QUININE SULPHATE PLUS SALICYL-SALICYLATE (SALYSAL) IN SOLUTIONS OF VARYING P_H

In each dialyzing sac were placed ½ gram of quinine sulphate, ½ gram of salysal and 20 c.c. of water of different P_H. Each sac was then placed in a solution of the same P_H.

TABLE V

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min.	$FeCl_3$ test	–	–	–	–	–	–	–
	Alk. ppt.	–	–	–	–	–	–	–
30 min.	$FeCl_3$ test	–	–	–	–	–	–	–
	Alk. ppt.	–	–	–	–	–	–	–
1 hr.	$FeCl_3$ test	–	–	–	–	+–	+	+
	Alk. ppt.	–	–	–	–	–	–	–
2 hr.	$FeCl_3$ test	–	–	–	–	+	+	++
	Alk. ppt.	+	–	–	–	–	–	–
3 hr.	$FeCl_3$ test	+	+	–	–	+	+	+++
	Alk. ppt.	++	+	+	–	–	–	–
6 hr.	$FeCl_3$ test	++	+	+	+	+	+	++++
	Alk. ppt.	+++	++	+	–	–	–	–
24 hr.	$FeCl_3$ test	++++	+++	+++	+++	++	+++	++++
	Alk. ppt.	+++	++	++	+	+–	–	–

The results are quite similar to those obtained with quinine bi-salicylo-salicylate.

TESTING FOR QUININE AND SALICYLATES IN ANIMAL'S BLOOD FOLLOWING THE ADMINISTRATION OF QUININE BI-SALICYLO-SALICYLATE BY MOUTH

The following tests were in the nature of trial methods for the detection of quinine bi-salicylo-salicylate or its cleavage products after the administration of this compound to animals.

1. A rabbit was given 1 gram of quinine bi-salicylo-salicylate by mouth. This was accomplished by means of a syringe and rubber tubing. About 200 c.c. of water were required to get all of the drug into the animal's stomach. Twenty hours later the animal was exsanguinated. After laking, the blood was shaken with several times the volume of chloroform. The chloroform was evaporated to dryness on a steam-bath; then portions of the residue were tested for quinine bi-salicylo-salicylate, quinine and salicylates. All were negative.

A portion of the hemolyzed residue from the chloroform extract was then tested for salicylates as follows: The hemolyzed blood was saturated with ammonium sulphate and rendered acid to about 0.6 per cent with H_2SO_4, brought to the boil, filtered, cooled, neutralized with NaOH solution, extracted with chloroform (4 times), evaporated and the residue from this was tested with $FeCl_3$ solution. A strong positive test for salicylates was obtained.

Another portion of the hemolyzed blood was tested for quinine according to the standard technic. The result was negative.

2. A rabbit weighing 2250 grams was given 5 grams of quinine bi-salicylo-salicylate by mouth in the manner indicated above. After eighteen hours the animal was exsanguinated and the blood tested for quinine bi-salicylo-salicylate, quinine and salicylates. Only salicylates were found. The urine likewise yielded only salicylates.

3. A large dog was given 10 grams of quinine bi-salicylo-salicylates at 2 P.M. on one day and at 11 A.M. the next day he was given another 10 grams. One-half hour after receiving the second dose the animal was bled. There

were 680 c.c. of blood obtained. An attempt was then made to recover unchanged quinine bi-salicylo-salicylate, quinine and salicylates from this blood by means of the well-known Stas-Otto process. We did not find the method practicable with so much blood, i.e., we could not get the product clear. We were obliged to finish up with ammonium sulphate acidified to about 0.6 per cent with sulphuric acid. A good test was obtained for salicylates only.

QUALITATIVE AND QUANTITATIVE ESTIMATION OF QUININE BI-SALICYLO-SALICYLATES AND ITS CLEAVAGE PRODUCTS (QUININE AND SALICYL) IN BLOOD

While performing the foregoing tests we found that the detection and estimation of quinine bi-salicylo-salicylates and its cleavage products, quinine and salicyl presented many difficulties. None of the standard methods was quite satisfactory. After many trials we finally evolved a satisfactory method. This method is a blend of several methods used in the estimation of quinine and salicyl plus some modifications of our own.*

Technic.—Five c.c. of blood are laked with 5 c.c. of distilled water in a small beaker, then 40 c.c. of saturated ammonium sulphate containing 0.6 per cent sulphuric acid are added to it and 5 grams of solid ammonium sulphate. The mixture is then heated on a moderately warm electric hot plate with constant stirring. After coagulation takes place the liquid is brought to the boil over a free flame, and immediately filtered through a Gooch crucible. The suction is continued until the mat is quite hard. Instead of using asbestos in the Gooch crucible, a single layer of high grade filter paper is more practicable. By means of a fine-bladed knife the contents of the crucible are transferred back to the beaker. The filter paper is washed with 3 or 4 c.c. of water, and a new paper is placed in the crucible. The coagulum in the beaker is macerated in the water used to wash the filter paper, then 40 c.c. of acid, saturated ammonium sulphate are added and the mixture is heated on the electric hot plate, then brought to the boil and filtered; this process is repeated four more times, only that in last three times it is preferable to use plain saturated ammonium sulphate.

The entire filtrate (about 250 c.c.) is transferred to a graduated flask and made up to volume. This solution can be used for the detection and estimation of quinine bi-salicylo-salicylate or for the detection and estimation of its cleavage products, quinine and salicyl. (The estimation of all three substances in the same solution is difficult to accomplish.)

1. *Estimation of Quinine.*—Prepare (1) a standard solution of quinine containing 1 part of quinine base (0.0134 gm. of quinine sulphate per liter was used) per 100,000 in saturated ammonium sulphate. (2) Prepare an acidified iodine solution. This is made by diluting 25 c.c. of N/10 iodine solution with 225 c.c. of N/10 HCl.

A definite amount of this acidified N/100 iodine solution is used with different concentrations of the standard quinine solution for comparative

*The most helpful method was that entitled "Estimation of Minute Quantities of Quinine in the Blood" by A. C. Roy, Ind. J. Med. Research 14: 129, 1926.

purposes. This test is very delicate. A difference of 0.001 mg. of quinine can easily be detected by means of a colorimeter.

For comparison with the unknown quinine solution a series of 10 tubes are made up as shown in Table VI.

TABLE VI

TUBE	QUININE SOLUTION C.C. OF STANDARD	C.C. OF SATURATED AMMONIUM SULPHATE SOLUTION
1	5.0	--
2	4.5	0.5
3	4.0	1.0
4	3.5	1.5
5	3.0	2.0
6	2.5	2.5
7	2.0	3.0
8	1.5	3.5
9	1.0	4.0
10	0.5	4.5

In another tube (all the tubes should be of the same size) 5 c.c. of the ammonium sulphate extract of the blood are placed. One-half c.c. of the iodine reagent is added to each tube. Then, the "unknown" tube is matched against the standard tubes. First roughly by means of the eye, then by means of a colorimeter with a light attachment. We found the Klett colorimeter very satisfactory.

It is desirable to compare the solutions immediately after mixing with the iodine solution as a turbidity develops on standing.

2. *Estimation of Salicyl.*—Four-fifths of the ammonium sulphate extract of the blood are placed in a separatory funnel and extracted with about 30 c.c. of pure ether. This process is repeated 3 or 4 times. The ether extract is placed in a large test tube (10″ × 1¼″) and evaporated by placing in warm water, the evaporation of the first portion of ether taking place as the second is being shaken with the ammonium sulphate extract, etc.

The final residue is dissolved in 15 c.c. of warm saturated ammonium sulphate solution. To 5 c.c. of this solution are added 10 drops of a freshly prepared solution of ferric ammonium sulphate (2 per cent) and then comparison is made against varying amounts of a standard solution of sodium salicylate. The standard solution contains 1-10,000 of salicyl and the solvent is neutral saturated ammonium sulphate.

Ten tubes containing varying amounts of the standard salicyl solution are prepared in the manner indicated for quinine and 10 drops of the ferric ammonium sulphate solution are added to each of these tubes. The tube containing the unknown amount of salicyl is then compared with these tubes by means of a Klett colorimeter (or other type provided with an electric lighting system).

NOTE.—The quinine may be estimated in a 5 c.c. portion of the above solution, but a greater amount of the iodine solution may be required to overcome the effect of the greater concentration of salicyl.

3. *Estimation of Quinine Bi-salicylo-salicylate.*—Quinine bi-salicylo-salicylate can be estimated by the method described for quinine, since it also gives a definite color with the iodine reagent. The standard solution in this case being a 1-100,000 solution of quinine bi-salicylo-salicylate dissolved in saturated ammonium sulphate solution. 1 c.c. of the iodine solution is added to each tube of standard and unknown solutions.

Unhydrolyzed quinine bi-salicylo-salicylate does not give the salicyl-ferric alum test.

RATE OF ELIMINATION OF THE CLEAVAGE PRODUCTS OF QUININE BI-SALICYLO-SALICYLATE FROM A NORMAL MAN

0.8 gram (12 grains) of quinine bi-salicylo-salicylate were taken at one dose by a normal adult man. There were 250 c.c. of water taken with the drug. The rate and duration of elimination of the cleavage products (quinine and salicyl) in the urine was then tested. The results are tabulated below.

TABLE VII

TIME	URINARY PRODUCT		TIME	URINARY PRODUCT	
10 minutes	Quinine =	−	12 hours	Quinine =	−
	Salicyl =	−		Salicyl =	++++
20 minutes	Quinine =	−	24 hours	Quinine =	−
	Salicyl =	−		Salicyl =	+++
30 minutes	Quinine =	−	30 hours	Quinine =	−
	Salicyl =	+−		Salicyl =	++
40 minutes	Quinine =	−	36 hours	Quinine =	−
	Salicyl =	+		Salicyl =	+
50 minutes	Quinine =	−	42 hours	Quinine =	−
	Salicyl =	+		Salicyl =	+
60 minutes	Quinine =	−	48 hours	Quinine =	−
	Salicyl =	++		Salicyl =	+
90 minutes	Quinine =	+−	54 hours	Quinine =	−
	Salicyl =	++		Salicyl =	+
2 hours	Quinine =	+	60 hours	Quinine =	−
	Salicyl =	+++		Salicyl =	+−
4 hours	Quinine =	++++	72 hours	Quinine =	−
	Salicyl =	+++		Salicyl =	−
8 hours	Quinine =	+			
	Salicyl =	++++			

It will be noted that the salicyl could be detected an hour before the quinine fraction and that it could still be detected for many hours after negative results were obtained for quinine.

TOXICITY AND FATAL DOSES OF QUININE BI-SALICYLO-SALICYLATE AS COMPARED WITH VARIOUS QUININE AND SALICYL MIXTURES

A large number of rabbits were used in the toxicity experiments but as the details are of little moment, only the average results will be cited.

All the animals were kept on the same diet, which was a full diet of bread and vegetables.

TABLE VIII

RÉSUMÉ OF TOXICITY TESTS

Drug given was quinine bi-salicylo-salicylate.	
½ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
¾ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
1 gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
1¼ gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
1½ gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
2 gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
Quinine sulphate + sodium salicylate equivalent in quinine and salicyl content to	
½ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
¾ gm. of quinine bi-salicylo-salicylate per kilo	toxic, nonfatal.
1 gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
2 gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
Quinine sulphate and salysal equivalent in quinine and salicyl content to	
½ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
¾ gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
1 gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
1¼ gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
Quinine salicylate equivalent in quinine content only to	
½ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
¾ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
1 gm. of quinine bi-salicylo-salicylate per kilo	toxic, nonfatal.
1¼ gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
Quinine salicylate + sodium salicylate equivalent in quinine and salicyl content to	
½ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
¾ gm. of quinine bi-salicylo-salicylate per kilo	nontoxic.
1 gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.
1¼ gm. of quinine bi-salicylo-salicylate per kilo	toxic and fatal.

SUMMARY OF THE ABOVE RESULTS

DRUGS	MAXIMAL TOLERATED DOSE GM. PER KILO	MINIMUM LETHAL DOSE GM. PER KILO	REMARKS
I. Quinine bi-salicylo-salicylate	1.00	1.25	
II. Quinine sulphate + sodium salicylate	0.75	1.00	Quinine and salicyl equiv. to that in quinine bi-salicylo-salicylate.
III. Quinine sulphate + salysal	0.50	0.75	Quinine and salicyl equiv. to that in quinine bi-salicylo-salicylate.
IV. Quinine salicylate	1.00	1.25	Quinine only equiv. to that in quinine bi-salicylo-salicylate.
V. Quinine salicylate + sodium salicylate	0.75	1.00	Quinine and salicyl equiv. to that in quinine bi-salicylo-salicylate.

It is interesting to note that the values in II and V are the same.

The temperature and respiration factors were also noted in the above experiments and the following data were observed:

1. In every case there was a drop in temperature following the administration of the drug, but no proportionality between the amount of drop and the dosage could be observed.

2. The respiration was very slightly affected with nontoxic doses. With toxic doses the respiration exhibited a marked increase. When lethal doses

were given the respiration markedly increased for several hours before death, followed by a decrease just before death.

THE ABSORPTION OF QUININE BI-SALICYLO-SALICYLATE AND ALLIED SUBSTANCES FROM THE STOMACH AND INTESTINES

The quinine bi-salicylo-salicylate was administered in the maximum tolerated dose (1.00 gm. per kilo). The other substances were given in amounts equivalent to the quinine content of quinine bi-salicylo-salicylate and, with the exception of the case of quinine salicylate, the salicyl content also equaled the amount present in this drug.

The drugs were introduced into isolated parts of the gastrointestinal tract, and in each case two hours later the blood was withdrawn and analyzed. The figures given in Table IX are the averages of several experiments:

TABLE IX

DRUG ADMINISTERED		QUININE FRACTION MG. PER 100 C.C. OF BLOOD	SALICYL FRACTION MG. PER 100 C.C. OF BLOOD
Quinine bi-salicylo-salicylate.			
	Gastric absorption	2.6	9.0
	Intestinal absorption	2.6	30.0
Quinine salicylate.			
	Gastric absorption	2.6	4.9
	Intestinal absorption	3.8	6.3
Quinine salicylate + enough sodium salicylate to make the salicyl equal to that of quinine bi-salicylo-salicylate.			
	Gastric absorption	2.7	22.0
	Intestinal absorption	3.2	49.1
Quinine sulphate + sodium salicylate.			
	Gastric absorption	3.0	28.0
	Intestinal absorption	3.3	35.3
Quinine sulphate + salysal.			
	Gastric absorption	2.3	10.3
	Intestinal absorption	2.6	25.4

One of the remarkable facts that the above figures exhibit is the constant coefficient of absorption of quinine regardless of the form in which it is administered. The absorption of the salicyl fraction is more variable. Another interesting point is the low coefficient of absorption of salicyl from quinine salicylate as compared with quinine bi-salicylo-salicylate. When sodium salicylate, however, was added to the quinine salicylate in sufficient amount to bring the salicyl content up to the quantity present in quinine bi-salicylo-salicylate the salicyl absorption was greater than in any other case.

CLINICAL OBSERVATIONS

It is, of course, to the clinical and bedside impressions that we owe our estimate of the value of any drug, hence, our clinical observations in regard to quinine bi-salicylo-salicylate, although limited in number, are not without interest.

Owing to the well-known fact that it is impracticable to make a detailed study of many upper respiratory infections, the only cases which were taken

for observation were those in which the temperature and other symptoms warranted confining the patients to bed.

The number of cases observed was 80. This series included 40 cases of acute tonsillitis, 20 of acute pharyngitis, and 20 cases of influenza.

The patients with acute tonsillitis exhibited in every case rather severe symptoms, namely, a temperature of 103° to 104.6° F., marked dysphagia, general body pains, and malaise. They all showed the usual signs of severe tonsillar infection, and in every case the bacteriologic report of the throat cultures was positive for streptococci. The initial blood counts of W.B.C. were from 12,000 to 18,000.

After 12 doses of 12 grains of quinine bi-salicylo-salicylate administered every two hours, marked relief was observed in the local and general symptoms; the temperature being normal in most cases. Thirty-six hours after the treatment was started the temperature of all the patients had returned to normal.

The 20 patients grouped under *acute pharyngitis* were also cases of the severer type. Their temperature ranged from 102° to 103.8° F. They all exhibited marked local reaction to the infective agent, and their initial blood counts of W.B.C. ranged from 9,000 to 11,000.

The twenty cases of influenza were of the respiratory, or febrile type. The sputum contained streptococci, or pneumococci and occasionally B. influenzae. The W.B.C. count was low. In thirty-six to forty-eight hours after the usual administration of quinine bi-salicylo-salicylate the temperatures were normal and the other symptoms were greatly ameliorated. All the signs of bronchial infection had cleared up in forty-two to seventy-two hours.

In none of the cases cited above was there any toxic reaction associable to quinine or salicylate.

On comparing this series with cases treated with other salicylates it was noticed that quinine bi-salicylo-salicylate acted more quickly in relieving symptoms and reducing temperature, and there was no accompanying gastric distress.

SUMMARY

From the various data that have been obtained from the foregoing experiments with quinine bi-salicylo-salicylate and related substances the following outstanding facts have been observed:

1. Quinine bi-salicylo-salicylate is very insoluble in neutral aqueous solutions.

2. In aqueous solutions of varying P_H it was found that quinine bi-salicylo-salicylate is most soluble in alkaline solutions of about P_H 10, next in acid solutions of about P_H 1, and least soluble in neutral solutions of P_H 7.

3. The dissociation fractions of quinine bi-salicylo-salicylate exhibit the chemical identification tests of quinine and salicyl.

4. The diffusion of quinine bi-salicylo-salicylate is greatest at P_H 10 and next at P_H 2.

5. Albumin does not interfere with the hydrolysis and diffusion of quinine bi-salicylo-salicylate.

6. The dialysis of quinine sulphate plus sodium salicylate from solutions of different P_H presents quite a different picture from the results obtained with quinine bi-salicylo-salicylate. In this case P_H 7 is the reaction point at which the salicyl fraction is most diffusible and the maximum diffusion takes place within thirty minutes. The quinine fraction is diffusible in acid medium only, and the diffusion starts after two hours.

7. Albumin has a greater retarding effect on the diffusion of quinine sulphate and sodium salicylate mixture than it does on quinine bi-salicylo-salicylate.

8. The experiments on the elimination of the cleavage products of quinine bi-salicylo-salicylate from normal human beings show that the salicyl fraction can be detected in the urine in about thirty minutes and continues to be eliminated for about sixty hours. The quinine fraction is first detected in about ninety minutes and ceases to be detectable after eight hours. The maximum elimination point of the two fractions is at about four hours.

9. The cleavage products of quinine bi-salicylo-salicylate (quinine and salysal) can be determined quantitatively. A method for their estimation has been devised.

10. The maximal tolerated dose and minimum lethal dose of quinine bi-salicylo-salicylate are greater than those of quinine sulphate plus sodium salicylate; quinine sulphate plus salysal; quinine salicylate plus sodium salicylate. In other words, quinine bi-salicylo-salicylate is less toxic.

11. Quinine bi-salicylo-salicylate and other quinine and salicyl mixtures caused a drop in body temperature when administered to animals, but no proportionality between the amount of drop and the dosage could be observed.

12. The respiration is very slightly affected with nontoxic doses of quinine bi-salicylo-salicylate and related products. With toxic doses the respiration exhibits a marked increase. When lethal doses are given the respiration increases appreciably for several hours before death, but just before death it decreases.

13. The coefficient of adsorption of quinine is remarkably constant when administered to rabbits in the form of quinine bi-salicylo-salicylate, quinine salicylate, quinine salicylate plus sodium salicylate, quinine sulphate plus sodium salicylate, and quinine sulphate plus salysal. The absorption of the salicyl fraction of these substances is much more variable.

14. All the distinctive characteristics of quinine bi-salicylo-salicylate combine to make it an unusually valuable drug, but, perhaps, the most important are its nonirritating effects in the stomach in moderately large doses, its relatively low toxicity and its high salicyl content.

The Department of Biological Chemistry of the Long Island College of Medicine wishes to thank Messrs. Merck & Co. of Rahway, New Jersey, for supplying the quinine bi-salicylo-salicylate and other chemicals, etc., used in the above research.

STUDIES IN THE ALIMENTARY CANAL OF MAN*

VIII. The Time Relationship of Gastric Peristalsis

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INTRODUCTION

IN OUR studies upon the activity of the human stomach as observed by roentgenoscopic methods we have repeatedly observed the initial phenomena of the appearance of peristalsis and have described it in the following terms. We make, quite naturally, the reservation that these phenomena do not invariably appear in complete expression and may indeed sometimes pass through successive phases so quickly as to be unrecognizable or apparent only as a foreshortened sequence with phases omitted.

When a five-ounce fluid meal at 70° F. is administered to a resting stomach the immediate consequence is transmission of contents right through the pylorus and the neutral waves (hunger contractions) are delineated. This immediate passage ceases however before the end of one minute. The exact duration of the passage and also of the neutral waves depends upon the type of meal swallowed. There is then a pause when neither movement of stomach nor passage of contents can be seen. After the pause indentations occur in the gastric wall. They are sometimes visible on both curvatures, depending upon the completeness of barium delineation but always appear along the greater curvature. They vary in number but occur typically at junction of pyloric canal with pyloric vestibule, at junction of pyloric vestibule with gastric tube, and midway along the gastric tube. After an appreciable pause these indentations begin to pulsate and then, after a varying interval, send off waves of peristalsis in the aboral direction. The first wave starts typically at the distal pulsation two minutes after the administration of the meal, the second at the intermediate and the third at the proximal pulsation. If conditions are favorable in that the upper gastric tube is sufficiently delineated, the fourth wave is seen to start high up in the gastric tube.

After the appearance of waves, one or more, it may be all of the pulsations disappear though their sites generally remain recognizable by a slight contraction of the greater curvature.

At any time any wave may disappear at a pulsation site and reappear, after a few seconds, at the next. This phenomenon is sometimes interpreted as an observation that a wave may start up at any pulsation site, not necessarily the highest. It is however perfectly true that in certain studies we have noted interpolated waves originating at divers sites along the lower gastric tube and the pyloric vestibule.

The regular rhythmic succession of waves may break down into apparently purposeless wavelets which we have defined as shimmer. There is no

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clear distinction between shallow indefinite peristalsis and shimmer. The latter simply represents the former without apparent force or directive purpose. It is most frequently seen after exhibition of a meal of low stimulative power like milk but there is also reason to believe that shimmer is sometimes induced by a subconscious nervousness perhaps unrecognizable by the patient. It is apt to follow a sudden touch on the abdominal wall by the observer's hand. It frequently replaces peristalsis early in the examination or when the patient is brought back to the screen after an interruption in the examination. It also is frequently seen to last for about half an hour after the administration of the meal in the first fluoroscopic examination undergone by the patient.

Temporary absence of peristalsis is itself probably often a subconscious nervous phenomenon for even in a practiced patient there may be no waves for a period of not more than two minutes from the commencement of roentgenoscopic examination when he is brought back to the screen after being disturbed for the making of a roentgenogram.

Compared with the amplitude of waves gastric peristaltic rhythm is rather constant in its time relations. In making serial roentgenographic studies for analysis by cinematographic technique, our routine, based upon this conclusion, calls for one picture every ten seconds. So regular in time relationship is the rhythm of peristalsis after an experimental meal that the ten-second interval in roentgenography records such differences in rhythm as may be found after different types of meal.

To make a proper analysis of peristaltic time relations requires the devotion of a roentgenoscopic session to this theme alone. Since the same stomach must be used in studying the influence of different meals the investigation must be spread over a considerable interval so that time may be given to the patient to recover from any possible effects of prolonged roentgenoscopy. Further the interval must be one during which the student can be reasonably assumed to enjoy steady good health and, in the evenness of a routine life, to be relatively free from fatigue and worry. Clearly such conditions can be fulfilled only in the summer vacation.

From such occasional observations as could be made in the course of roentgenoscopic examinations designed for other purposes we estimated the time taken in passage of a wave along the stomach to the pylorus as approximately the following: from junction of pyloric vestibule and canal, 7 secs.; from mid-vestibule, 16 secs.; from junction of gastric tube and pyloric vestibule, 26-27 secs.; from mid-gastric tube, 35 secs.; from Magenblase (fundus) 60-62 secs. It was intended to replace these by more accurate determinations when opportunity should arise. Having had this chance to secure the necessary data we are now able to present the facts which are indeed essential for the adequate further prosecution of our gastric investigations.

TECHNIQUE

The method followed has been to administer a large meal in order to define the stomach outline as high up the gastric tube as possible and also to maintain the clearness of definition as long as possible. It has been found that, after some meals at least, buttermilk for example, there is progressive in-

crease in peristaltic amplitude during the first ten minutes. We have also shown to our satisfaction that entry of the stomach into the rather quiescent neutral phase, characteristically commencing twenty minutes after a five-ounce meal, is much delayed by exhibition of a large meal. We have therefore administered large meals consisting of sixteen ounces of vehicle and four ounces of barium sulphate. Roentgenoscopic observations began, in each study, fifteen to thirty minutes after the meal was swallowed. One of us (W.A.S.) acted as the subject of these experiments and was entirely responsible for working up the data; another (W.M.K.) made the time determinations and checked the records set down by the third (T.W.T.) who is responsible for the final form of this manuscript. The initial observations upon which this study was planned were carried out by W.M.K. and checked by T.W.T. The subject is quite experienced in roentgenoscopic examinations and has had many investigations made upon his own stomach, including two serial studies. His age, when the study commenced, was 21 years, his height 1747 mm. and his weight 207 pounds. The conditions of health and routine mentioned above have been properly fulfilled. The observer (W.M.K.) sits in front of the roentgenoscopic screen and notes the first appearance of a wave of which the progress is to be followed. She calls out the site where the wave is first noted and the exact time is registered by the recorder (T.W.T.) who has, before him, a stop-clock with a second-hand. As the observer traces the progress of the wave she calls out the arrival of the wave in each successive segment or level of the stomach. The times are duly recorded to the nearest second and thus the time relationship of the wave is registered. This method was adopted after first attempting to make the record directly by means of a dictaphone and with the aid of a radium-faced stop-clock. The manoeuvers necessary to watch roentgenoscopic screen and stop-clock at the same time proved too complicated and the time was better recorded in the manner described as finally adopted.

In order to avoid injury to the subject, each roentgenoscopic session has been limited to the registration of approximately ten waves and some time has been allowed for the subject to recover before the next session was scheduled.

In this study we decided to compare the time relationship of peristalsis after six large fluid meals. These are water; milk; buttermilk; lactic acid solution in water buffered with sodium hydroxide to pH 4; sp. menth. pip. 20 minims in sixteen ounces of water; and sodium bicarb. 60 grains (4 grams) in sixteen ounces of water. Thus each large meal consisted of sixteen ounces of fluid together with four ounces of barium sulphate. Each meal is given at a temperature of 70° F. since it has been shown that raising or lowering the temperature of the gastric contents modifies the peristalsis.[3] The average time-relationships have been calculated and the standard deviations computed from the formula derived by "Student" for a very small series.[2]

The effects of application of heat and of cold to the abdominal wall upon gastric behavior pattern were also included in our study and are recorded. A hot pack or an ice-bag was held to the abdominal wall for a period of forty-five minutes. A twenty-ounce milk-barium meal was administered thirty minutes after the pack or bag was first applied to the abdominal wall and

fifteen minutes before the examination. Then, the subject standing behind the roentgenoscopic screen, the pack or bag was removed, and the gastric behavior pattern watched at frequent intervals for half-an-hour.

The eight studies here recorded were spread over two successive summer vacations in order that there might be no possible detrimental effect from prolonged and often-repeated x-ray exposures and also that the mental and physical health requirements laid down above might be maintained.

In our subsequently recorded observations the gastric subdivisions used are the junctions of gastric tube with pyloric vestibule and of pyloric vestibule with pyloric canal. The gastric tube and pyloric vestibule are each subdivided into upper, middle and lower sections and the time registered when the wave passes over the center of each.

RECORDS OF GASTRIC BEHAVIOR, CHEMICAL STIMULI

The qualitative observations made at the successive sessions are important for establishing the entirely regular behavior of the stomach under examination. The dimensions of the gastric shadow depend not so much upon the amount of the experimental meal as upon the interplay of pyloric action and gastric secretion called forth. Water, for example, results in an immediate profuse gastric secretion but, owing to continued patency of the pylorus which, after water, in our experience, never completely closes, the stomach shadow remains relatively small. The water-stomach shows a motor response which is relatively more evanescent than those consequent upon other fluid stimuli. According to our observations a five-ounce water meal is flushed from the stomach by gastric juice in about ten minutes whereas all the other fluids, studies of which are here recorded, betray their presence by a characteristic response for about twenty minutes after administration. We started the observations upon effect of water stimulation about twenty-five minutes after administration of the large meal and continued them until fifty minutes after administration in order to note the effect of the flushing process upon the wave time as the water became more diluted and finally displaced by the gastric secretion. In this we were disappointed since the method of recording does not permit such fine distinction but the standard deviation in Table I gives a rough measure of change of wave time.

Water meal. The entire stomach outline was clearly seen and the gastric tube very narrow, consistent with the patent pylorus above mentioned. Several waves were present at one time, giving an impression of unusual speed. The waves were of considerable amplitude, obvious on both curvatures and almost pinched the barium shadow in two.

In Table I may be seen the average length of time which the wave takes to reach the pylorus from each gastric level beyond the fundus, together with the variability in this time. It is important to observe, first, that, after water, waves can be traced usually from mid-gastric tube and occasionally from upper gastric tube. Secondly the speed of waves in a water stomach is greater than after any one of the other five fluid stimuli with the exception, in the pyloric part, of peppermint. The speed of water-stimulated peristalsis is therefore a standard against which to check the peristalsis induced by other forms of stimulation.

TABLE I.

TIME IN SECONDS TAKEN BY WAVE TO TRAVEL TO PYLORUS FROM DIFFERENT GASTRIC LEVELS WITH STANDARD DEVIATIONS

LEVEL	PEPPERMINT			WATER			MILK			BUTTERMILK			SODA			LACTIC ACID		
	NO. WAVES TIMED	MEAN	S. D.	NO. WAVES TIMED	MEAN	S. D.	NO. WAVES TIMED	MEAN	S. D.	NO. WAVES TIMED	MEAN	S. D.	NO. WAVES TIMED	MEAN	S. D.	NO. WAVES TIMED	MEAN	S. D
Upper GT	1	44.0	—	1	39.0	—	—	—	—	2	49.0	—	1	41.0	—	—	—	—
Middle GT	5	35.4±1.34	2.80	7	32.0±0.72	2.14	—	—	—	3	40.6	—	6	38.1±1.76	5.23	—	—	—
Lower GT	1	29.0	—	7	27.1±0.82	2.42	2	29.0	—	8	32.3±0.81	2.69	2	32.0	—	—	—	—
Jn. GT & PV	10	25.8±0.70	3.28	11	23.4±0.61	2.99	7	24.5±0.90	2.66	11	28.0±0.61	2.37	10	27.5±0.96	4.55	10	30.6±1.21	4.76
Upper PV	9	17.7±0.95	3.46	11	17.5±0.53	2.61	9	18.0±0.45	1.62	10	20.3±1.24	3.46	9	21.6±0.71	2.61	11	23.1±0.81	4.01
Middle PV	10	11.9±0.58	2.74	13	12.2±0.45	2.42	11	13.0±0.29	1.41	10	12.4±0.52	1.89	10	15.1±0.53	2.50	11	15.3±0.58	2.87
Lower PV	10	7.3±0.16	1.89	13	8.0±0.49	2.63	11	8.3±0.24	0.96	9	8.5±0.43	1.57	10	10.0±0.31	1.45	11	12.7±0.67	3.31
Jn. PV & PC	10	3.7±0.41	1.85	12	4.5±0.20	1.04	11	4.4±0.16	0.77	10	5.2±0.35	1.36	10	5.4±0.13	0.66	12	5.8±0.58	2.48

GT: Gastric tube; PV: Pyloric vestibule; PC: Pyloric canal; Jn: junction.

TABLE II

AVERAGE TIME IN SECONDS FOR PASSAGE OF WAVE FROM ONE GASTRIC LEVEL TO THE NEXT

	PEPPERMINT	WATER	MILK	BUTTERMILK	SODA	LACTIC ACID
Upper GT Middle GT	8.0	5.0		10.5	3.0	
Lower GT Jn. GT and PV	7.0	4.9		8.0	7.0	
Upper PV Middle PV	5.0	4.0	7.0	4.0	5.5	6.0
Lower PV Jn. PV and PC	7.9	6.0	6.5	5.6	7.0	7.2
Pylorus	6.6	5.4	5.3	7.5	6.0	7.0
	4.6	4.2	4.7	3.8	5.1	5.3
	3.6	3.8	3.9	3.0	4.6	5.5
	3.7	4.5	4.4	5.2	5.4	5.8

Number of observations and verbal contractions as in Table I.

Table II gives the average time in seconds occupied by a wave passing from one gastric level to the next. This table has been built up from averages of the actual records and not by subtraction of one average of Table I from another. Roughly the intervals are five seconds except in distal pyloric vestibule and pyloric canal when, the distances being shorter, the intervals are less. The water-stimulated wave passes regularly along the stomach without pauses such as are observed in peristalsis stimulated by other contents. The water-waves do not weaken as they proceed, do not fade out temporarily or break down into shimmer.

Milk Meal.—When the stomach was first observed, twenty minutes after the meal, the barium had settled and the gastric outline was clearly apparent from pylorus to upper pyloric vestibule. The Magenblase was flat and the gastric tube shadow relatively wide in comparison with its diameter after the water meal. This difference in gastric tube width is due to secretion of gastric juice stimulated by the milk but held in the stomach as a result of the pyloric rhythm which brings about alternate opening and closure of the pylorus in place of the practically continuous patency after water administration. Milk waves are shallow and very apt to break down into shimmer. A faint shadow of intermittent passage through the pylorus was noted.

Reference to Table I demonstrates that there is no difference in speed between milk-stimulated waves and water-stimulated waves except in the lower gastric tube. Statistically one would not say that there is a difference even here. But experience assures us that speed is less rapid about the junction of gastric tube and pyloric vestibule than in the pyloric area when the peristaltic waves are milk-stimulated. Indeed we had difficulty in observation at the milk session, through waves fading out about this level or breaking down into shimmer or even being obscured and inhibited in a pulsating ring at the junction of tube and vestibule.

From Table II one learns that the loss of time resulting from this reduced speed averages about three seconds. Our unpublished records also show a faint suggestion of the retardation carrying over into upper pyloric vestibule.

Buttermilk Meal.—When the observations were first made twenty-five minutes after the meal the entire stomach outline was clearly visible. The gastric tube was very wide as is usual after buttermilk owing to the rapid outpouring of gastric juice. Further this great breadth of gastric tube was not due to a simple anteroposterior compression of the stomach, for we turned the patient side-ways and observed that the gastric tube shadow was the same breadth from before backwards as from side to side. The stomach was adjusted to approximately the same distance from the screen in both views. The usual dense shadow of passage was noted after every wave.

Table I shows a marked reduction in speed of waves through the gastric tube compared with the speed of water- and milk-stimulated waves, but no such slowing up in the pyloric part. It is apparent that the slight pause or slackening of speed noted about the junction of tube and vestibule in the milk stomach was but the tail-end of the reduction of speed in gastric tube which we have now had the opportunity to observe after buttermilk. The amplitude of milk-stimulated waves is very small but that of the waves after water or buttermilk is quite marked. Hence speed and amplitude have no relation to each other. The difference between milk waves and buttermilk waves is more clearly seen in amplitude: the difference between buttermilk waves and water waves is rather one of speed. It is not to be assumed however that we intend to make any special distinction in pattern of rhythm between gastric tube and pyloric vestibule. Slowing of the rhythm is best marked and earliest noted at the fundic end of the stomach and spreads aborally. After a milk meal the slowing is confined entirely to gastric tube but, as Tables I and II both show, after a buttermilk meal the speed tends to slow down in vestibule also.

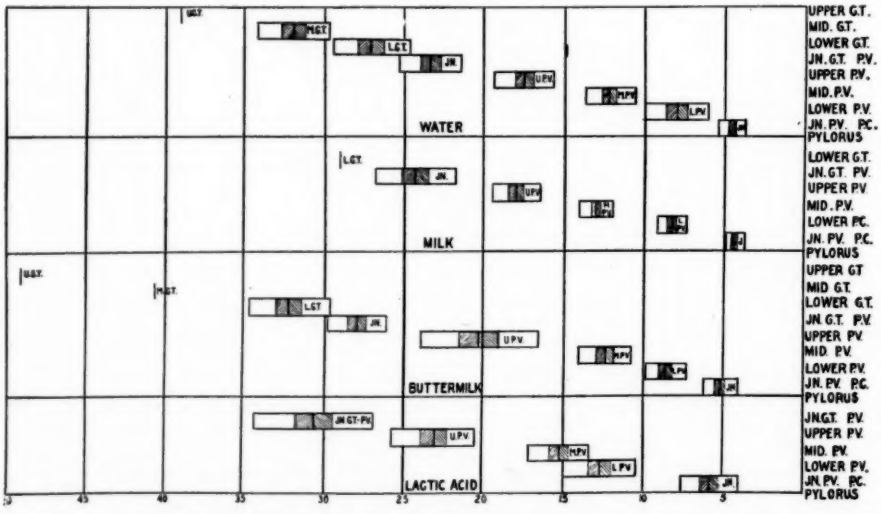
Lactic Acid Meal.—Quarter of an hour after taking the meal the gastric tube was moderately broad from secretion of gastric juice and waves could be observed, deep and numerous, along the greater curvature. Unfortunately, gas in the splenic flexure prevented us from making observations proximal to the junction between gastric tube and pyloric vestibule. However this handicap is not so severe as it might seem because the record indicates a slowing of rhythm all the way along the stomach so far as our observations were possible. Both Tables I and II indicate this general slackening of speed which, upon the basis of the buttermilk pattern, may be inferred also in the gastric tube where conditions of the experiment denied us direct observations.

Reference to Table I emphasizes the erratic time-relationship of lactic acid waves. The standard deviation is usually about twice that of the buttermilk waves at identical gastric levels. This is but one example of the character of the lactic acid behavior pattern. We have been greatly puzzled to interpret our observations on lactic acid. In some stomachs the behavior simulates that of buttermilk; the gastric shadow, especially in the tube, is large: the waves are slow and their amplitude great. In other stomachs the lactic pattern is more nearly that of water: the gastric shadow is narrow with both ballooning and cog-wheel effects so typical of water: the waves are short and rapid and their amplitude moderate.

Soda Meal.—It was not until the following summer (1930) that the remaining experiments here recorded were undertaken. With so long an inter-

val as a year elapsing between the two groups of experiments it was necessary to insure that the general condition of the stomach had not changed in the interval. We therefore used our "outliner" to give us an observation of gastric activity before starting the experiment. This outliner consists of five grams of barium sulphate in half an ounce of water. It is just enough to outline the greater curvature and delineate the waves without affecting them for more than five minutes, since this small quantity of water is very rapidly flushed through the pylorus by secretion of gastric juice.

Having ascertained that the condition of the stomach was unchanged from the previous summer, we administered a large soda meal, the composition of which has been stated. In this subject the entire gastric tube and pyloric vestibule were distended, as frequently, but not invariably, occurs after the exhibition of this drug. Gas in the splenic flexure interfered with



Figs. 1 and 2.—Graphic presentation of the phenomena recorded in Tables I and II.

The distances of the central thick lines from the right hand ordinate represent the average time taken by a wave to travel to the plyorus from that particular gastric level. Verbal contractions as in Table I.

The hatched areas indicate the probable error on each side of the mean and the unshaded rectangle delimits the value of the standard deviation. The progressive slackening of speed in the milk, buttermilk, lactic acid and soda series is very clearly shown.

observations upon the waves in the upper gastric tube. The waves however were quite definite, deep, forceful, slow soda waves.

Reference to Table I shows that the progress of the soda wave is relatively slow throughout its entire course. The speed of passage over the stomach wall is very similar to that of buttermilk and the variability in time of successive waves is again very similar to that of buttermilk. In form, however, the waves could not be mistaken for buttermilk waves for their outline is undulating and forceful rather than constricting and vigorous.

Peppermint Meal.—On the day after the soda pattern was studied, a large peppermint meal, of the composition noted above, was given after a regular outliner had been administered to identify the gastric response. The Magenblase, gastric tube and pyloric vestibule remained rather narrow as our studies have led us to expect after peppermint administration. The resulting

behavior, as illustrated in Table I, is somewhat peculiar. In the pyloric vestibule and canal the speed of peppermint waves is practically that of water, but in the gastric tube, the waves are slow, forceful and deliberate. In our earlier observations we considered that peppermint waves were indistinguishable from those induced by soda. This conclusion was due to an emphasis of the strongly marked forceful waves so obvious in the gastric tube. In variability, peppermint waves resemble those of buttermilk. They are less regular than water waves but much more regular than those of lactic acid.

Reference to Table II emphasizes the apparent pause of the wave in the junction between gastric tube and pyloric vestibule which is seen in lactic acid and soda patterns but occurs most markedly in the peppermint response where it would almost seem that the wave-rhythm changes between upper and lower parts of the stomach.

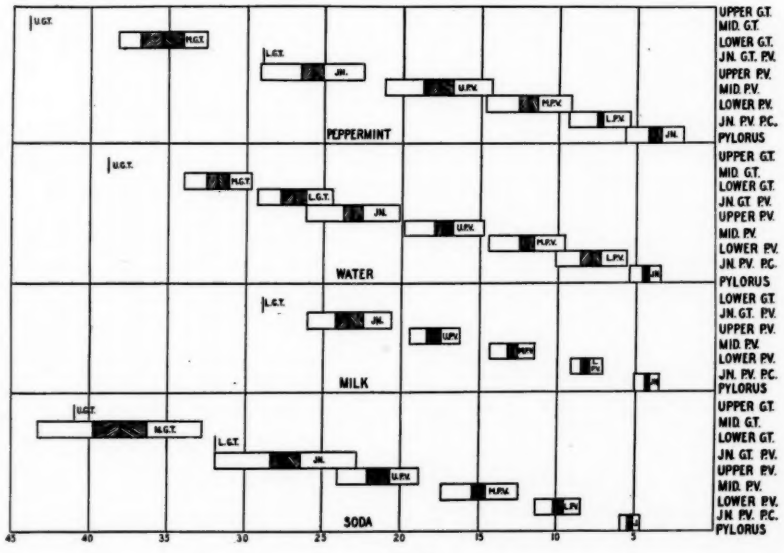


Fig. 2.

The above observations on peppermint and soda in this particular trained and stabilized stomach must be considered as of a different category from those upon water, milk, buttermilk and perhaps upon lactic acid. Irregularities in pattern suggest the influence of dilution. Further studies are needed.

EFFECT OF HEAT AND COLD

The observations on heat and cold were undertaken in the early fall of 1930. In the earlier part of this essay the method of preparation of the patient for these studies has been fully described. The method of studying the effect of heat and cold is bound to differ from that of the chemical excitants which have been described above. It is characteristic of the physical stimulus of heat or cold that the effect is most marked at first and gradually becomes less with a lapse of time. After the exhibition of a chemical stimulus the effect increases and is always at its height between five and ten minutes after the experiment begins. In an earlier article[3] we have pointed out that

the effect of both heat and cold is an increased gastric activity as shown by amplitude of waves and rapidity of peristalsis. The effect of cold is much shorter in duration than that of heat. Whereas the depth, speed, frequency and vigor of peristalsis induced by heat may continue in diminished degree for fifteen or even twenty minutes, the stimulant effect of cold has already faded at the end of five minutes.

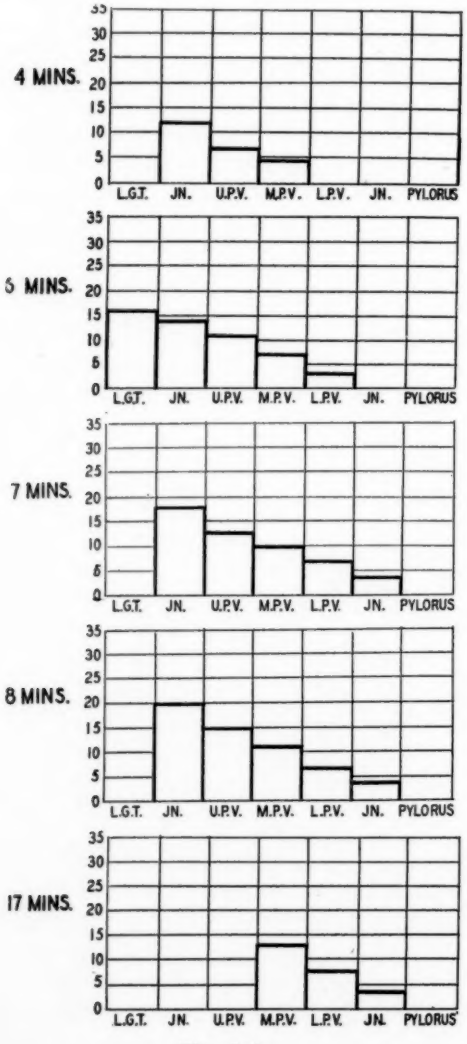


Fig. 3-A.

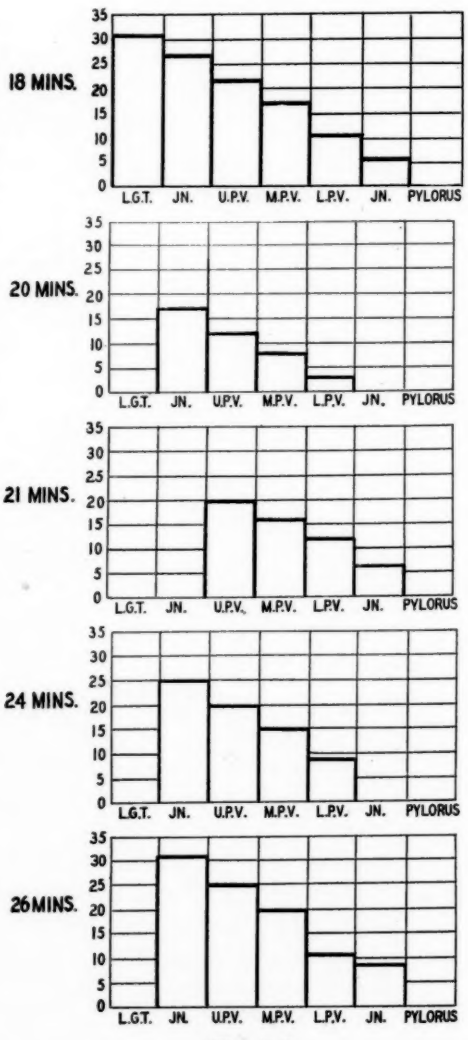


Fig. 3-B.

Fig. 3.—Serial record of gastric waves after the application of external heat.

Four minutes after cessation of heat application to abdominal wall the speed is still three times that of normal water-stimulated waves. The speed progressively diminishes until about eighteen minutes after removal of stimulus. Abscissae in seconds. Verbal contractions as in Table I.

The technique of observation was essentially different from that in the foregoing studies. We were not now concerned in the variability of a rather constant type of rhythm which indeed developed to a maximum at some minutes after the observations began. We now had to face a rhythm, the initial

expression of which would be most typical and vigorous and the later exhibition less typical in appearance. Although we could not prolong the experimental session we made observations at approximately every two minutes after the withdrawal of the hot pack or the ice-bag from the abdominal wall and have recorded these in Figs. 3 and 4. In order to facilitate comparison

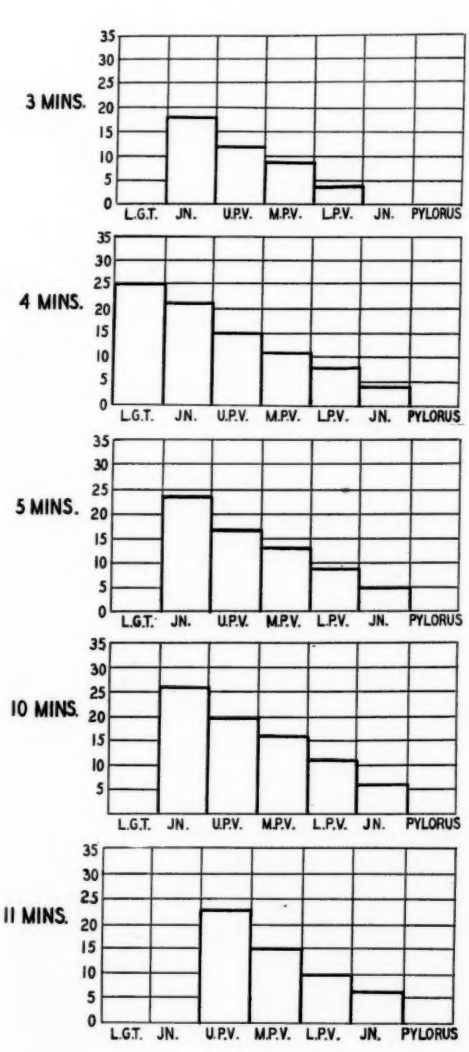


Fig. 4-*A*.

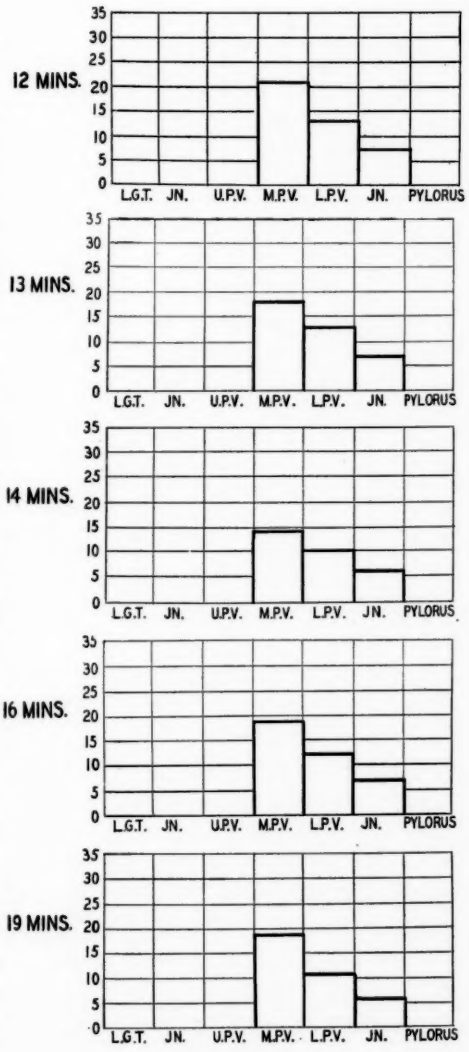


Fig. 4-*B*.

Fig. 4.—Serial record of gastric waves after application of external cold.

Five minutes after cessation of cold application to abdominal wall the speed originally induced by cold is reduced to that of ordinary milk waves. A further reduction in speed occurs until the expiration of ten minutes. Abscissae in seconds. Verbal contractions as in Table I.

with the effect of chemical stimulation we have introduced Table III which gives the total average length of time taken by the wave induced by the different chemical stimuli to reach the pylorus from the successive gastric levels. Table III is directly comparable with Figs. 3 and 4.

TABLE III

COMPARISON OF WAVE TIMES TO PYLORUS IN SECONDS

	UGT	MGT	LGT	JN	UPV	MPV	LPV	JN
Peppermint	44.0	35.5	29.0	26.0	17.5	12.0	7.5	3.5
Water	39.0	32.0	27.0	23.5	17.5	12.0	8.0	4.5
Milk			29.0	24.5	18.0	13.0	8.5	4.5
Buttermilk			32.5	28.0	20.5	12.5	8.5	5.0
Soda	41.0	38.0	32.0	27.5	21.5	15.0	10.0	5.5
Lactic Acid				30.5	23.0	15.5	12.5	6.0

Owing to the difficulties in observation it was not always possible to examine the wave throughout the course of the stomach. Our records in Figs. 3 and 4 are therefore somewhat patchy; they do however give a quite clear impression of the fading effect of heat or cold upon the gastric mechanism.

The reader may wonder why we applied heat and cold to the abdominal wall instead of making use of hot or cold drinks. In our previous article[3] we have pointed out that the indirect or reflex effect of heat and cold through the abdominal wall is more vigorous and typical than that obtained by the exhibition of hot and cold drinks.

Effect of Heat.—For several minutes after the observations were begun we were unable to time the gastric waves, the passage of which, at least in the lower part of the stomach, was very rapid. Four minutes after the observations began the passage of a wave from junction of gastric tube and pyloric vestibule to pylorus took only twelve seconds as against twenty-four seconds for water or milk. The speed of the heat wave along the pyloric vestibule was so great that four seconds only were occupied in passing from mid-vestibule to pylorus as against the twelve seconds characteristic of water, milk, peppermint and buttermilk. Thereafter, the speed of the heat waves diminished until about eighteen minutes after the observations began, at which time, as indicated in Fig. 3, the speed was practically that of milk at 70° F. It is true that occasional irregularities appear as illustrated in the wave recorded twenty minutes after commencement of the study.

We continued this examination for twenty-six minutes and found that, during the last ten minutes of the examination, the gastric waves became unusually slow. It is not fair to state that this was due to the supervention of the neutral pattern. In the first place the neutral pattern does not assert itself so rapidly in a stomach containing a large meal. In the second place the time relationship of neutral waves is practically that of the water pattern. One must therefore conclude that, after eighteen minutes, the vigor of gastric peristalsis induced by heat gives place to a rather lethargic rhythm.

Effect of Cold.—The examination of the gastric pattern reflexly stimulated by application of cold to the abdominal wall follows exactly the lines of that adopted for heat. We were faced with the same difficulty at first, namely, that the speed of the early waves was so great that they defied exact time measurement. At the end of three minutes however, the time taken by a wave to pass from the junction of gastric tube and pyloric vestibule to pylorus was eighteen seconds as against the twenty-four seconds taken by milk or water waves to travel the same distance. From the mid-pyloric vestibule the wave occupied eight seconds instead of twelve or thirteen. This activation by cold

is then much less vigorous than that induced by heat. An inspection of Fig. 4 demonstrates the rather rapid diminution in speed of the waves induced by cold so that, at the end of five minutes from the commencement of observations, the time relationship is practically that of milk at 70° F. Thereafter, until some eleven minutes after removal of the cold pack, there was a progressive slowing in the wave time. During the remainder of the study the time relationship of gastric waves remained unchanged.

CONCLUSIONS

Our series of eight sessions has helped us considerably to understand the phenomena of gastric behavior. It is true that we have had only one stomach under special observation. But this was a carefully trained and stabilized organ whose general behavior pattern we knew from repeated study under different conditions. Moreover the results of our investigation of this stomach confirm and extend the occasional quantitative observations which alone have been possible to us in the past.

The water-stimulated stomach should be taken as the standard because its behavior is practically that of the neutral organ; because wave-amplitude is great and waves can be followed from upper gastric tube with ease; and also because the wave travels with a steady rhythm throughout its course and does not tend to fade out or break up into a shimmer.

The milk, buttermilk and lactic acid groups of observations form a definite series, but in amplitude milk waves are very shallow and without the vigorous character of buttermilk waves or the erratic vigor of the lactic acid waves. The series is one of rhythm progressively slackening. Milk-stimulated waves are slightly slower in their speed of passage over gastric tube but progress in pyloric vestibule is exactly similar to that of water-stimulated waves. After buttermilk this slackening of speed is continued into the vestibule. After lactic acid it becomes general and marked throughout the stomach.

The soda waves, apart from their deep and forceful character, are similar to those of buttermilk in their time relationship. These peppermint waves, on the contrary, have approximately the neutral character in common with those of water and of milk, throughout the pyloric part of the stomach. They are however definitely slow in the gastric tube and seem to pause more distinctly than those of soda and of lactic acid at the junction of gastric tube and pyloric vestibule.

The waves induced by heat are at first vigorous and very rapid. The effect is not lost until the lapse of about eighteen minutes after removal of the hot pack. Thereafter the time relationship is lengthened as though the waves become lethargic in character. Waves stimulated by cold diminish in speed so rapidly that five minutes after removal of the cold pack their distinctive character is lost. They continue to diminish in speed until the end of about ten minutes when they become stabilized.

Further study is needed to elucidate the cause of these differences in gastric behavior pattern and further observations are essential in order to establish their actuality. We are recording these results now because we feel convinced, in the light of our experience, that the phenomena herein de-

scribed are definite and will be confirmed by careful observers upon trained and stabilized stomachs.

SUMMARY

The rhythm of gastric peristalsis is definitely influenced by the particular stimulus which initiates it. Water produces a regular active peristalsis.

Milk-stimulated waves differ from those produced by buttermilk and lactic acid in being of low amplitude, but in rhythm they fall into series with the waves of buttermilk- and lactic acid-stimulated peristalsis.

Milk-stimulated peristalsis is slightly slowed in speed in the gastric tube but not in pyloric vestibule. The speed of buttermilk-stimulated waves is slightly slackened in pyloric part as well as in gastric tube. But the lactic acid meal produces waves very definitely reduced in speed throughout their progress from upper gastric tube to pylorus.

Soda waves are deep and forceful and their time relationship is very similar to that of buttermilk-stimulated waves.

These peppermint waves, like water and milk waves, are almost indistinguishable in time relationship from the neutral pattern in the pyloric part of the stomach. They are easily distinguished from the neutral pattern by their greater amplitude and vigor. In the gastric tube they are distinctly slow like the soda waves.

The waves stimulated by soda, peppermint and lactic acid appear to pause at the junction between gastric tube and pyloric vestibule.

The influence of heat and cold differs essentially from that of the chemical stimuli above recorded in that the initial effect is the most powerful and there is a constantly diminishing degree of expression whereas the effect of a chemical stimulus rises to a maximum a few minutes after its earliest expression.

The waves induced by heat are at first so rapid that the record of their time relationship is very difficult. At the end of four minutes the speed is still about three times that of water-stimulated waves. The speed progressively diminishes until about eighteen minutes after the heat stimulus has been removed. The stomach then apparently enters a lethargic phase.

The effect of cold is similar to that of heat but far less pronounced. By the end of five minutes the speed of waves induced by cold has become reduced to that characteristic of an ordinary milk meal at 70° F. Until the end of ten minutes there is a progressive slowing in the wave time but thereafter the time relationship is unchanged.

REFERENCES

Instead of referring to definite paragraphs of previous articles from this laboratory, a very difficult and involved procedure, we give a list of references to the more important contributions bearing upon the problem in hand. A general summary has been given of our observations to date by the senior author in his recent book on the Behavior Patterns of the Alimentary Tract published in 1930 by Williams and Wilkins, Baltimore.

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AN ANALYSIS OF FIFTY HEART CASES SHOWING LOW VOLTAGE*

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A REVIEW of the literature has shown conflicting reports as to the significance of electrocardiograms of low voltage, that is, with R waves of 5 mm. or less in all leads. Master and Pardee[1] stated that a voltage of the QRS group so low that its size does not exceed five mm. in the lead of largest excursion is a significant abnormal finding. Hepburn and Jamieson,[2] in a series of 34 patients, stated that there is no doubt as to the gravity of low voltage electrocardiograms as a prognostic sign, and that the only other group with a higher mortality is that of bundle-branch block. Sprague and White,[3] in a study of 57 patients, stated that they had never observed electrocardiograms of low voltage in patients with normal hearts. They concluded that it is a finding of diagnostic and prognostic importance in forming an opinion of the myocardial ability of any individual. Willius and Killins[4] reviewed 140 patients presenting electrocardiograms of low voltage. All their electrocardiograms showed low voltage alone; records displaying other significant abnormalities were rejected. They stated that it is not justifiable to conclude that electrocardiograms of low voltage unassociated with other graphic abnormalities indicate serious myocardial disease or are of serious prognostic importance.

During the past three years, approximately two thousand electrocardiograms have been taken on the Medical Service of the Cincinnati General Hospital. These tracings have not been taken routinely on all patients, but only on those in whom there was either definite or questionable evidence of heart disease. Of this number, 50 cases showed low voltage, and our study comprises a review of these 50 cases in detail. It is to be noted that these patients on whom these electrocardiograms were taken were practically all from the laboring class and as is usual with patients of this class, many of them showed evidence of marked heart failure on admission to the hospital.

The criterion which we have used for the diagnosis of low voltage was that the total deflection of the QRS complex did not exceed 5 mm. in any of the three standard leads. All the records were taken on an amplifying instrument with the usual standardization, that is, one centimeter deflection equivalent to one millivolt current.

Of these 50 patients, 47 entered the hospital because of symptoms referable to the heart. Their ages varied between twenty and eighty with about an equal distribution in each decade. There were 36 males and 14 females. The majority of the patients were white. Shortness of breath and edema of

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the extremities were the leading symptoms. The duration of the illness was less than one year in 33 of the patients.

Arteriosclerotic heart disease was the etiologic factor in 30 of these patients, 16 of them having an associated hypertension. Two others showed hypertension alone. Seven were rheumatic, and there was one each of the following: Infectious, syphilitic, combined syphilitic and arteriosclerotic. One patient had a carcinoma of the lung with metastases to the pericardium and myocardium. In four, tuberculosis was the etiologic factor, two showing pericarditis with effusion and the two others an adherent pericardium, as proved at autopsy. In two, the etiology was unknown. In only one case was there no clinical evidence of heart disease.

The heart was enlarged in 42 of the patients. In 43 there was no valvular disease. Mitral stenosis was present in three patients, aortic insufficiency in two and mitral insufficiency in two. Pericardial effusion was present in 5 patients. Adherent pericardium was found at autopsy in three cases and was diagnosed clinically in two others. There was heart failure of the congestive type in 47 cases and of the anginal type in one other. Only four patients showed pulsus alternans. There were no cases of myxedema in our series.

We have separated the electrocardiograms showing low voltage alone from those in which low voltage was associated with auricular fibrillation, auricular flutter, inverted T-waves in the significant leads, aberrant QRS complexes, including incomplete bundle-branch block, any degree of heart-block or ectopic tachycardias. There were 19 patients showing low voltage alone. Of this number 16 are dead, two are untraced and one is still living. Of the number dead, 10 died within one month after the first electrocardiogram, showing low voltage and all were dead within a period of nine and one-half months.

Auricular fibrillation was associated with low voltage in 14 patients. Eight are dead, four are untraced and two are still living. Five died within one month after low voltage was first discovered and the other three died within ten months.

The QRS complexes showed aberration in all leads in 10 cases, 4 of which are classified as incomplete bundle-branch block. Of these, 9 are dead and one is untraced. Six died within one month, the remaining 3 within ten and one-half months.

Heart-block, including delayed conduction, partial or complete block, was associated with low voltage in 9 cases. Six of these are dead, 4 within one month and 3 are still living.

Seven cases showed inverted T-waves in the significant leads. Three died within two and one-half months, 3 are still living and one is untraced.

Twenty-five of these patients had more than one electrocardiogram. In 10 of these, the low voltage persisted in all tracings. In 8 others the voltage later returned to normal. Tracings had been taken in 7 before low voltage appeared. In 19 of these cases we observed that there was a correlation between the patient's condition and the voltage of the electrocardiogram, in that the voltage was greater as the patient's condition improved. In 6 other cases there was no such relationship. In one case with pericardial effusion the

voltage returned to normal following the removal of 900 c.c. of fluid from the pericardium.

There were 36 deaths in the 50 cases which comprised this series. Seven of the patients are known to be living and 7 are untraced. Two of those known to be living are still in the hospital. Of the 36 deaths, 32 occurred within six months from the time the first electrocardiogram showing low voltage was taken. Twenty-six of these patients died in the hospital and autopsy was performed on 14. Heart failure was the cause of death in 26.

In the 14 autopsies* the heart was found to be enlarged in 10. Pericardial effusion was found in 4, adherent pericardium was present in 3. The coronary arteries showed some degree of sclerosis in 10 cases. Microscopic examination showed abnormalities in the heart muscle in every case, the most frequent finding being fibrosis of the myocardium. This was present in 9 cases. Arteriosclerosis was present in 8 cases. All the cases with pericardial effusion showed coronary arteriosIcerosis, myocardial fibrosis, or both.

SUMMARY

1. Forty-seven of the 50 patients showing low voltage gave symptoms referable to the heart. The duration of the illness was less than one year in 33. Forty-nine had clinical evidence of heart disease.

2. Arteriosclerosis, with or without hypertension, was the leading etiologic factor in our series.

3. In 19 cases low voltage alone was present. The mortality in this group was 16 or 84 per cent within ten and one-half months.

4. Fourteen cases showed auricular fibrillation in addition to low voltage. The known mortality of this group was 8 or 57 per cent within ten months.

5. Ten patients showed aberrant QRS complexes, including intraventricular block. The known mortality of this group was 9 or 90 per cent in ten and one-half months.

6. Nine patients showed heart-block. Six or 67 per cent were dead in seven months.

7. Seven patients showed inverted T-waves in significant leads. Three or 43 per cent were dead within two and one-half months.

8. There were 36 deaths in the series of 50 cases; 32 of the patients died within six months from the time the first electrocardiogram showing low voltage was taken.

9. Autopsies were performed on 14. The coronary arteries showed some degree of sclerosis in 10 cases. Microscopic examination showed the myocardium to be abnormal in every case, fibrosis of the myocardium being the most frequent finding.

CONCLUSION

We believe from the study of the above 50 cases that the occurrence of a voltage of 5 mm. or less regardless of other electrocardiographic abnormalities in a patient with heart disease is of serious prognostic import.

*We are indebted to Dr. Pearl M. Zeek of the Department of Pathology of the University of Cincinnati and the Cincinnati General Hospital for the autopsy reports.

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LABORATORY METHODS

A CRITICAL STUDY OF THE POLYNUCLEAR COUNT AS ADVOCATED BY SCHILLING*

By E. M. Medlar, M.A., M.D., Mt. McGregor, N. Y.

THE increasing interest in the Schilling method of the differential counting of the leucocytes has led the author to a critical study of the method in tuberculous cases. The method advocated by Schilling[1] is a somewhat simplified Arneth[2] method. Both authors have recognized and emphasized the fact that a considerable amount of useful information may be gained from differential leucocytic counts if one takes cognizance of the variations in nuclear architecture of the neutrophile. Both authors have stressed the fact that the more the neutrophiles are representative of the immature stage of that cell the more serious is the infection.

The most serious drawback to the method of both of these authors is that it is rather difficult to identify accurately all of the neutrophiles as they have classified them. A considerable difference in personal judgment enters into any detailed classification such as they advocate. Realizing the value of this method, others have sought to further simplify it. The most significant contributions toward a simplified classification have been made by Pons and Krumbhaar,[3] Piney,[4] Cooke and Ponder,[5] and Farley, et al.[6]

Our study was concerned with the changes in the nuclear variations of the neutrophile from day to day to determine, if possible, the real significance of the younger cells in the circulation. We were interested to find out whether a marked increase of young forms in one count over those in a previous count would indicate a more serious condition of the patient. To simplify the classification down to a minimum we have placed all nonsegmented nuclear forms of the neutrophile into Class I, and all segmented forms, regardless of the number of segments, into Class II. With this modification the only consideration is whether the nucleus is segmented or nonsegmented and the differences in personal judgment are reduced to a minimum. The following table will show in what way this classification has condensed that of the authors mentioned above. This simplified classification is the same as advocated by Farley, et al.

Table I

	CLASS I	CLASS II
Arneth	I	II, III, IV & V
Schilling	I, II & III	IV
Pons and Krumbbaar	I & II	III

*From the Hegeman Research Laboratory of the Metropolitan Life Insurance Company Sanatorium.

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Our study included 100 individuals who were patients in the sanatorium. Repeated counts were done on each individual. The smallest number of counts made on any case was four. In the large majority of individuals ten to twenty counts were done. Our study extended over a period of six months and about 1,200 counts were made. All of the differential counts were made by the author in order to have as uniform results as possible and to eliminate the factor of judgment on the part of others.

The blood smears were made on cover-slips and were stained with Wright's blood stain. Four hundred cells were counted in every instance. The total leucocytic counts were done in the usual way from a one-to-twenty dilution of the blood in 0.1 per cent acetic acid solution.

The majority of the cases in our study were proved cases of tuberculosis. We had the opportunity, however, to include in our observation individuals who were ill from diseases other than tuberculosis.

We have selected from our entire group nineteen cases to illustrate the leucocytic pictures which we have encountered in various diseases and in different stages of tuberculosis. Table II gives the results of our observations.

In Table II the first three cases show what we would consider a normal distribution between the young and the segmented forms of the neutrophile. These cases agree with the findings of Schilling who has established that about 4 per cent of the total leucocytes are of the "band" form. We would consider that the nonsegmented forms might be as high as 7 or 8 per cent without real pathologic significance. If the immature forms are maintained above 8 per cent, the neutrophile count is abnormal and the greater the percentage of nonsegmented forms the more serious is the pathologic condition.

Case G. E. (acute appendicitis) shows that although there was a mild leucocytosis and a high percentage of neutrophiles the nonsegmented forms were not increased above normal. Operation was performed within twelve hours. Histologic examination of the appendix showed extensive neutrophilic infiltration of the tissues with but little exudate free in the lumen of the organ.

Case E. S. shows a normal total leucocytic count except on the day of death when a marked leucocytosis was present. Note the marked and persistent shift in the neutrophiles to the nonsegmented forms. The count done on the day of death showed 70 per cent of all leucocytes to be nonsegmented neutrophiles. This case, as well as several others in the table, agrees with the observation of Schilling that the total leucocyte count gives much less reliable information than the differential leucocytic count, if the latter is properly done and correctly interpreted. One might be led to the conclusion that the bone marrow in the case was incapable of causing a leucocytosis; that it was exhausted. That this was not true is shown by the agonal leucocytosis with a tremendous outpouring of immature forms. Myelocytes were frequently present but it was not necessary to have a greater subdivision of the neutrophile types to give a clear understanding of the seriousness of the pathologic process.

Case L. B. demonstrates that a decided shift to the immature neutrophiles occurs during the height of a positive smallpox vaccination reaction.

TABLE II

CASE	REMARKS	DATE	TOTAL COUNT	NEUTROPHILES		LYMPHOCYTES	MONONUCLEARS	EOSINOPHILES	BASOPHILES
				NON-SEG.	SEG.				
Ca.	No disease.	7/31	5 300	5	56	30	6	3	0
		8/ 4	6 100	4	52.5	32	10	1	0.5
		8/11	7 700	5	41	39	10	4	1
		8/12	6 900	4	45	36	10	4	1
		8/15	4 500	3	46	39	10	2.5	1.5
Dw.	Malnutrition.	7/29	6 400	3.5	53	28	9	5	1.5
		8/12	8 200	5	55	25	8	6	1
		8/18	9 900	3	55	28	7.5	6	0.5
		8/25	7 700	5	56	25	8	4.5	1.5
A. L.	Incipient pulmonary tuberculosis.	6/20	6 700	3	49	38	7	2.5	0.5
		8/ 7	7 000	3	52	37.5	6	1.5	0
		8/14	8 300	4	56	31	6	2.5	0.5
		8/21	8 200	5	54.5	31	8	1.0	0.5
G. E.	Acute appendicitis (normal convalescence after operation).	7/26 A.M.	13 000	8	82	7	3	0	0
		7/26 P.M.	13 500	7	80	8	5	0	0
		8/ 6	7 400	5	70	16	7	1.5	0.5
		8/28	5 600	5	61	21	11	1	1
E. S.	Streptococcus viridans. Endocarditis with septicemia. Died August 2.	6/17	5 100	33	50	10	5	1	1
		6/19	6 600	43	44	7	4	2	0
		6/23	8 200	53	35	7	4	1	0
		6/24	9 000	47	38	9	5	0.5	0.5
		7/28	6 600	43	44	7	4	2	0
		7/30	8 000	45	39	9	7	0	0
		8/ 2	23 900	70	20	5	4	1	0
L. B.	Chronic bronchitis. Positive "take" following smallpox vaccination.	12/ 4	6 100	12	44	19	21	2	2
		12/ 5	5 700	23	42	13	20	1	1
		12/ 8	7 000	37	40	9	13	0.5	0.5
		12/ 9	6 600	25	37	17	18	2.5	0.5
		12/12	7 100	14	44	24	16	1.5	0.5
		12/15	8 400	6	46	27	17	2	2
		12/18	7 600	8	55	17	17	2.5	0.5
		12/22	6 800	9	49	23	15	3	1
		12/23	4 600	8	49	26	14	1.5	1.5
		12/26	5 700	8	54	24	12	1.5	0.5

TABLE II—CONT'D

CASE	REMARKS	DATE	TOTAL COUNT	NEUTROPHILES		LYMPHO-CYTES	MONO-NUCLEARS	EOSINO-PHILES	BASO-PHILES
				NON-SEG.	SEG.				
Ho.	Carcinoma of lung.	6/19	7 100	9	60	13	14	3	1
		8/ 4	6 600	7	63	14	11	4.5	0.5
		8/11	6 200	14	58	11	13	4	0
		8/18	6 700	10	62	13	12	3	0
		8/25	5 400	11	55	25	7	1.5	0.5
		9/ 2	5 500	10	62	11	12	4	1
X.	Incipient pulmonary tuberculosis.	6/23	5 800	6	54	30	8	1.5	0.5
		8/19	3 800	3	46	39	10	2	0
		8/26	3 900	4	44	40	10	1.5	0.5
		9/ 3	4 800	7	46	32	10	4	1
		9/ 9	5 200	6	48	37	6	2.5	0.5
	Acute gastrointestinal upset due to indiscretion in beverage.	9/11	10 000	21	66	7	5	0	1
		9/12	5 500	13	45	32	7	2.5	0.5
		9/13	3 600	8	40	37	12	3	0
De L.	Far advanced pulmonary tuberculosis. Died Nov. 20, 1930.	5/15	9 400	37	39	11	10.5	1.5	1
		5/16	14 700	30	45	9	11	1.5	0.5
		5/19	8 700	33	44	8	13	1	1
		5/20	12 500	31	53	5	10	1	0
		5/22	10 200	21	50	11.5	16	0.5	0
		5/23	10 000	27	54	6.5	12	0.5	0
		5/26	10 400	29	50	8	12	1	0
		5/27	9 900	33	47	6	12	1.5	0.5
		5/29	8 000	24	45	11	18	1.5	0.5
		5/30	11 200	30	47	11	10	1.5	0.5
		6/ 2	8 400	32	45	10	12	1	0
		6/ 3	5 400	37	45.5	7	10	0.5	0
		6/ 5	8 000	38	41	6	13	1	1
		6/13	10 000	22	56	11	10	1	0
		6/16	14 900	24.5	50	10	11	4	0.5
		6/17	12 000	30	46	11	10	2	1

TABLE II—CONT'D

CASE	REMARKS	DATE	TOTAL COUNT	NEUTROPHILES		LYMPHO-CYTES	MONO-NUCLEARS	EOSINO-PHILES	BASO-PHILES
				NON-SEG.	SEG.				
Lo.	Far advanced pulmonary tuberculosis. Died Nov. 1, 1930.	5/15	12 800	17	60	10	11.5	1	0.5
		5/16	10 600	13	62	12	11	1	1
		5/19	7 900	13	66	9.5	11	0	0.5
		5/20	8 800	14	61	14	10	1	0
		5/22	7 000	14	60	15	10	1	0
		5/23	8 900	10	65	13	11	1	0
		5/26	6 900	18	58	11	12	0.5	0.5
		5/27	9 300	11	60	18	10	1	0
		5/29	8 300	12	62	11	13	1	1
	Abscess of tooth with marked swelling of face. Tooth extracted. Prompt recovery.	5/30	12 500	27	58	7	7.5	0	0.5
		6/ 2	9 500	20	52	14	14	0	0
		6/ 3	7 600	15	58	14	11	1	1
		6/ 5	8 300	11	61	14	11	2	1
		6/13	7 400	13	70	10	6.5	0.5	0
		6/16	7 700	10	65	13	11	0.5	0.5
		6/17	8 300	9	66	13	10	1.5	0.5
Ca.	Far advanced pulmonary tuberculosis. Has been a strict bed patient for years.	6/17	12 600	10.5	72	9	7	1	0.5
		6/20	12 200	15.5	65	14	4	1.5	0
		6/27	14 000	12	72	12	3	1	0
		7/ 4	10 800	28	51	15	6	0	0
		7/11	13 000	13	65.5	13	6	2	0.5
Re.	Far advanced pulmonary tuberculosis.	7/28	7 800	14	61	14	9	2	0
		8/ 4	7 900	15	57	17	8.5	2	0.5
	Hemorrhage and acutely ill.	8/29	11 000	35	53	4	7.5	0.5	0
		9/ 3	13 000	20	61	8	10	0.5	0.5
		9/10	9 600	25.5	58	8	8	0.5	0
Dic.	Moderately advanced pulmonary tuberculosis.	6/20	7 200	8	47	29	14	2	0
		8/ 1	6 300	8	55	27	8	1.5	0.5
		8/12	6 100	15	49.5	24	9	2	0.5
	Blood-streaked sputum and x-ray evidence of "spread" of tuberculous process.	8/19	7 200	18	44	24	12	2	0
		8/22	5 400	12	53	21	11	2	1
		8/27	5 500	28	37	17	16	2	0
		9/ 2	6 300	25	43.5	18	12	1	0.5
		9/ 6	7 300	21	47.5	16	12	2.5	1
		9/16	5 000	18	39	23	16	3	1

TABLE II—CONT'D

CASE	REMARKS	DATE	TOTAL COUNT	NEUTROPHILES		LYMPHOCYTES	MONONUCLEARS	EOSINOPHILES	BASOPHILES
				NON-SEG.	SEG.				
Sch.	Moderately advanced pulmonary tuberculosis. Has a large basal lesion. Patient has shown considerable clinical improvement during sanatorium residence.	5/12	10 500	11	51	22	12	3	1
		5/15	9 300	12	51	19	14	3	1
		5/16	6 400	8	54	20	14	3	1
		5/19	8 500	10	50	23	13	3.5	0.5
		5/20	9 000	9	52	22	14	2.5	0.5
		5/22	9 000	4	53	25	15	2.5	0.5
		5/23	8 700	7	50	26	12	4.5	0.5
		5/26	9 000	6	55	20	15	3	1
		5/27	9 500	10	51	23	13	2.5	0.5
		5/29	8 000	7	55	21	12	4	1
		5/30	6 800	9	51	23	13	2.5	1.5
		6/ 2	7 100	9	49	22	13	6.5	0.5
		6/ 3	6 900	10	49	23	13	4	1
		6/ 5	9 100	9	53	24	11	2.5	0.5
		6/13	9 400	7	57	19	11	5.5	0.5
		6/16	9 600	8.5	54	23	9	5	0.5
		6/17	5 800	8	55	20	12	4	1
Bo.	Far advanced pulmonary tuberculosis. Artificial pneumothorax, pneumolysis and phrenicectomy.	5/29	8 100	6	60.5	24	8	0.5	1
		6/ 5	7 300	5	60	28.5	8	1	0.5
		6/19	9 600	4	58	32	4	2	0
		6/24	7 400	4	57	31	6	1.5	0.5
		7/31	9 000	7	64	19.5	8.5	1.0	0
	Increased exercise before discharge from Sanatorium.	8/ 7	8 300	16	56	14	13	1	0
Sw.	Far advanced pulmonary tuberculosis Artificial pneumothorax. Very little clinical improvement.	8/ 1	17 000	8.5	66	16	9	0.5	0
		8/ 8	15 000	12	70	10	8	0	0
		8/15	11 000	15	62.5	13	9	0	0.5
		8/22	12 300	10	71	11	8	0	0
		9/ 5	19 000	12	69	12	7	0	0
		9/30	16 500	9	70	10	9	1.5	0.5
		10/28	15 300	13	66	13	7	0.5	0.5

TABLE II—CONT'D

CASE	REMARKS	DATE	TOTAL COUNT	NEUTROPHILES		LYMPHOCYTES	MONONUCLEARS	EOSINOPHILES	BASOPHILES
				NON-SEG.	SEG.				
Br.	Moderately advanced pulmonary tuberculosis. Artificial pneumothorax. Clinical symptoms improved.	5/27	7 600	7	60	17	9	6	0
		6/ 3	21 000	12	64	11	12	1	0
		6/16	7 400	8	59	20	7.5	5	0.5
		6/23	12 000	11	61	15	6	6	1
		7/30	8 900	17	58	15	5	4.5	0.5
		8/ 4	6 100	11	54	20	11	3.5	0.5
		8/11	8 500	12.5	54	18	8	7	0.5
		9/ 1	8 400	13	64	11	8	4	0
		9/30	9 400	6	62	17	11	3	1
Ste.	Moderately advanced pulmonary tuberculosis. Artificial pneumothorax. Good clinical progress.	8/ 6	7 800	5	56	29	7	2	1
		8/13	9 000	5	60	23	8	2	2
		8/20	6 600	5	54	30	8	2	1
		8/27	8 500	3	50	33	10	3	1
		10/ 1	7 400	3	56.5	28.5	9.5	2	0.5
		10/ 6	9 500	6	60	22	10	1.5	1.5
		10/13	8 300	7	58	22	11	1.5	0.5
		10/20	10 500	5	58	25	10	1	1
		10/28	10 000	2	49	37	9	1.5	1.5
Sta.	Far advanced pulmonary tuberculosis. Artificial pneumothorax. Excellent clinical progress.	5/30	10 200	2	53	35	8	1.5	0.5
		6/ 6	4 500	2.5	51	34	9	2.5	1
		6/13	7 300	1.5	53.5	32	10	1.5	1.5
		6/20	5 800	2	56	32	8.5	1	0.5

This promptly receded with the subsidence of the pathologic process. Here again the differential count gives information which would be missed entirely if the total count alone was relied upon.

Case Ho. demonstrates that the shift in neutrophilic forms can occur in malignancy. Again a normal total count is present.

Case X. shows that this same shift toward immature neutrophiles can occur when there is no evidence of infection. The significance of this will be emphasized in the discussion. In this case a total leucocyte count of 10,000 is evidence of a leucocytosis for he normally runs between four and six thousand in his routine counts.

Case De L. was a case of extensive and progressive pulmonary tuberculosis who had been seriously ill for three years before death. Considerable variation occurs in the number of immature neutrophiles from time to time but they are always well above the normal percentage. Marked fluctuations also occur in the total counts. There is no correlation between the total leucocytic count and the percentage of immature neutrophiles.

Case Lo. was very similar to Case De L. The percentage of mature forms of neutrophiles ranges higher and the immature forms lower than in Case De L. Note the marked increase of immature neutrophiles at the time of the acute inflammation of the facial tissues and the prompt subsidence following extraction of a diseased tooth. Although the immature forms are less in this case than in Case De L. the percentages of all neutrophilic forms are about the same in both instances. Death occurred sooner in this case than in Case De L.

Case Ca. is another serious case of tuberculosis, the patient having been bed-ridden for over three years. Note the fluctuations in the nonsegmented forms and the tendency to maintain a high level in segmented forms of neutrophiles.

Cases Re. and Dic. are included to show that a marked increase of nonsegmented neutrophiles occurred with a clinical exacerbation of the tuberculous process. Note that this increase has persisted since the first rise was observed. In case Dic. this increase in immature forms antedated the clinical exacerbation of the disease.

Case Sch. shows that a case with a large basal pulmonary lesion need not exhibit a constant shift in the neutrophile forms to the nonsegmented types. This is of real significance since it informs one that the neutrophiles are not in excessive demand.

Case Bo. is included to illustrate the effect of increased physical activity upon the leucocytic reaction. This is a case in which artificial pneumothorax, pneumolysis and phrenicectomy failed to control the progress of the disease. The last two counts were done just prior to her discharge from the Sanatorium and show a marked rise in immature neutrophiles on the last count.

In case Sw. there has been a persistent leucocytosis. The neutrophiles were increased above normal in both the nonsegmented and segmented forms. Even where there is a constant leucocytosis the greatest increase of immature neutrophiles does not occur with the highest total count. Artificial

pneumothorax has not changed the leucocytic picture and thus indicates the inefficiency of the treatment in this particular case.

Case Br. is quite similar to Case Sw., except that here we do not have a persistent leucocytosis.

Cases Ste. and Sta. are two individuals in whom artificial pneumothorax has brought about a very marked shift in the differential leucocytic picture from the neutrophile toward the lymphocyte side. Such a change was noted in doing the differential counts in the usual way without separation of the neutrophiles into distinct groups. At the time the counts, included in Table II, were done both cases were improved clinically. The leucocytic picture, taken as a whole, is consistently better in Case Sta. In neither case is there an increase in nonsegmented neutrophiles.

DISCUSSION

Farley, et al., state that "the test of a given clinical procedure is often the ease of its application divided by its usefulness." We wholly agree with this statement. Any laboratory method which can be of routine value should be reduced to the minimum of work consistent with giving as much information as possible. In special instances an elaboration of the simplified method may be justifiable but it is doubtful whether the added information gained will render a much more accurate insight into the biologic process being studied. While one always desires scientific data to be minutely accurate, still in biologic processes one is interested more in the limits of variation which may be considered within normal and in the significance of the variations beyond normal limits. In any instance one should not be too dogmatic about the limits set for normal. It should be recognized that fluctuations above or below normal will occur which may have no clinical significance. It is only when such fluctuations beyond a normal range persist that serious attention should be given them. From this one can readily see that a single differential leucocytic count is insufficient. With a simple technic more counts and hence more information may be gained with the same amount of labor used in a more elaborate method.

Most physicians are interested in leucocytic counts as diagnostic aids in infectious processes. We are of the opinion that the leucocytic response in pathologic processes extends far beyond the domain of infections. While abnormal leucocytic reactions are found consistently in infections of any severity, it should be borne in mind that such a finding is but one phase of a general biologic rôle played by the various leucocytes. Noninfectious lesions, such as infarction of heart muscle, severe burns, intraabdominal hemorrhage, etc., may cause as marked an alteration in the leucocytic picture as an infectious process. It would seem more logical to regard a leucocytic response of whatever type as indicative of the type of alteration in the tissues to which the various leucocytes respond than to insist that an infection must be present whenever an abnormal leucocytic picture is encountered.

We have included Case X. in Table II to show that one may encounter a marked increase of immature neutrophiles as a response to a noninfectious lesion. The illness in this case was the result of the indiscreet choice of an

alcoholic beverage. To us this case demonstrates the fallacy of attempting to determine the presence or absence of an infection by the leucocytic response found. That the leucocytic picture will be of great aid in determining the severity of the process, whether infectious or noninfectious, is unquestionable. The prompt return of the leucocytic formula in this case to normal was quite striking.

There is quite a tendency among physicians to rely more upon the total than upon the differential leucocytic count. The cases included in this paper show that one cannot rely upon the total count alone as an index of an abnormal leucocytic picture. A mild leucocytosis may occur with a normal differential count or one may encounter a marked shift in the numbers of the different cell types with a normal or even a low total count. If it is possible to have only a total or a differential count then it is better to choose the differential count to obtain information. However, both the total and differential counts should be obtained wherever possible for together they give more information than either alone.

The classification of the leucocytes as stressed by the authors mentioned in this paper has had a tendency to emphasize very strongly the neutrophile and to lay less stress upon the other cell types. This is commendable to a degree for in those pathologic processes most incompatible with life of the tissues the neutrophile plays the leading rôle. However, in evaluating any leucocytic count one should always note the proportions of the different cells, for each type has its own significant part to play. This can best be appreciated by comparing the counts in Case De L. with those in Case Sch. In previous papers[7, 8] we have dealt in detail with the significance of the different leucocytic types in tuberculosis, and it would seem to be unnecessary repetition to comment further upon this subject here.

During the past several years we have made a very extensive study of the leucocytes in various diseases. We have used a classification in which we did not attempt to divide the neutrophiles into nonsegmented and segmented forms, although we had noted an increase of the nonsegmented forms very commonly in patients who were seriously ill. From our present study we are led to the belief that it is hardly necessary to have the elaborate subdivision of the neutrophiles if one appreciates fully the normal mode of the leucocytic types. Another factor which has come to our attention is that one must not place too great importance upon the extent of the shift in the neutrophile types. It will be noted that in our cases there has occurred marked fluctuations from day to day. The significant and very important point is that in the patients who were seriously ill the nonsegmented neutrophiles were consistently above normal.

If one considers the presence and the extent of the shift in the neutrophilic forms as more significant than the total number of all neutrophiles then erroneous conclusions may be drawn. For instance Case G. E. had practically no increase in nonsegmented forms although her total neutrophiles were markedly increased. To have awaited a shift in the neutrophilic types before operation was advised would have in this case caused an inexcusable delay.

It will be noted in some of the cases included in Table II that there is

but little if any difference in the total number of neutrophiles, although there is considerable difference in the number of nonsegmented forms. (Compare Cases De L., Lo. and Ca.) We have observed that in a number of serious tuberculous cases there occurred an abnormally high percentage of segmented neutrophiles with only a slight to a moderate increase of nonsegmented forms. To conclude that those cases with a high percentage of nonsegmented forms are more critically ill than those with a moderate increase would be erroneous, as is shown in Cases De L. and Lo. It would appear probable that in some individuals there has occurred a sufficient expansion of the hematopoietic tissue in the bone marrow so that mature neutrophiles can be produced rapidly enough to meet the demand and hence one does not find a high percentage of the immature cells in the circulation. From our study it seems to us that the shift in neutrophilic types signifies more the ability of the marrow to produce mature neutrophiles in sufficient number than it does the seriousness of the pathologic process. In acute pathologic conditions, such as lobar pneumonia, the sudden demand for neutrophiles does not allow time for the hematopoietic tissue to sufficiently expand to meet the demand with mature cells, hence, a very high percentage of nonsegmented neutrophile types in the circulation. In chronic progressive tuberculosis there is time for the expansion of the hematopoietic marrow tissue and if this expansion has occurred one may find only a slight increase of the immature forms.

In the Schilling method great importance is placed upon the presence of the most immature neutrophiles, viz., the metamyelocyte and the myelocyte. Wherever there is an excessive demand for neutrophiles one would expect a few of the most immature forms to be present in the circulation. In any cell type, where great pleomorphism is shown in the developmental cycle, too much stress should not be given to any one phase of the process. We agree with Piney that Schilling's Classes I and II may very readily be included in his Class III for routine clinical work. The same may be said of Class I of Pons and Krumbhaar.

A criticism that may be made relative to differential leucocytic counts as done in a large number of places is that but one hundred leucocytes are counted. The author considers this an insufficient number, for no matter how expertly the blood films are made the different leucocytic types will not be evenly mixed. It is probable that they are not evenly mixed in the circulating blood. We feel that four hundred cells should be counted in every differential count in order that the percentage of the leucocytic types of smaller numbers may be more nearly correct. If a high dry magnification of four hundred is used one may classify four hundred cells as accurately and almost as rapidly as one hundred under oil immersion.

Farley, et al., state that as high as 16 per cent of nonfilament neutrophiles may be regarded as normal. If such be the case then a very high proportion of our counts cannot be considered pathologic. Since 65 per cent of their normal counts have 10 per cent or less of nonfilament neutrophiles, we believe it justifiable to consider the remainder abnormal. It is not uncommon to find single abnormal counts in individuals who are considered healthy. Here we wish to stress again, as we have in a previous paper,[9] that one must

not place too great reliance on a single leucocytic count. The persistence of abnormality in repeated counts is the thing of real significance.

SUMMARY

We believe that, if one desires to divide the neutrophiles into different groups in the differential leucocytic count, it is sufficient for practical purposes to establish but two classes, nonsegmented and segmented. Such a classification often gives more striking proof of the abnormality of the leucocytic picture than the grouping of all neutrophiles together. However, in the majority of instances, one need not have this subdivision of the neutrophiles to appreciate the fact that an abnormal leucocytic reaction is present.

We consider, from our studies, that a persistence of more than 8 per cent of nonsegmented neutrophiles is abnormal. The sustained increase rather than the increase in a single count is the point of importance.

To attempt to diagnose infections by any leucocytic count we regard as fallacious, for noninfectious tissue lesions can cause a leucocytic reaction identical to that caused by an infectious agent. In either instance the leucocytic response simply indicates the type and severity of the tissue damage.

In many instances an abnormally high percentage of neutrophiles occurs without any or with but slight increase in nonsegmented forms. Such a leucocytic reaction should be given as careful consideration as instances where there is a marked shift to the nonsegmented forms. This reaction we have observed in early acute appendicitis, proved by operation, and in chronic progressive tuberculosis. It would seem that the shift to immature forms depends very largely upon whether the amount of leucopoietic tissue is sufficient to produce sufficient segmented mature neutrophiles to meet the demand. The change in the neutrophilic forms may indicate the volume of leucopoietic tissue the individual case possesses more than the seriousness of the pathologic process.

We regard single leucocytic counts and the counting of but 100 cells in the differential as insufficient in any case where leucocytic studies are deemed of value.

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A SIMPLE THERMOCOUPLE AND A THERMOPILE FOR DETERMINATION OF TEMPERATURE IN BIOLOGY AND MEDICINE*

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THE thermocouple is a very sensitive type of thermometer and with proper care will indicate minute changes in temperature quickly and accurately. It is adaptable also to various special requirements of a problem, for instance: it can be made either flexible or rigid, and it is possible to make the sensitive point very small and insert it like a needle into tissues. The temperature of situations difficult of access can often be determined with the thermocouple and readings taken at any desired distance away.

An additional feature which is sometimes valuable is that any number of individual points or junctions can be connected to the main thermocouple and used in various locations at the same time. A continuous record of temperature is possible with the aid of accessory apparatus.

The thermocouple is comparatively easy to make and equally simple to use. If the details of construction and operation were more generally known this excellent method of reading temperature might be more widely used. Following is a description of the construction and use of a thermocouple and a thermopile, and of the principles involved in the method.

THE PRINCIPLE OF THE THERMOCOUPLE

If wires of any two dissimilar metals, copper and nickel for instance, are fused or soldered end to end, a "thermoelectric" junction will be formed at each connection. A thermocouple consists essentially of two such junctions connected into a circuit, as shown at *A* and *B* in Fig. 1-a. The junctions are called thermoelectric partly because one of the dissimilar metals has a greater number of electrons than the other or the electrons have greater energy values; and consequently a flow of electricity will take place from one metal to the other. This results in an "electromotive force" (e.m.f.) being set up at each junction, and the magnitude of this force depends upon the temperature of that junction.

The electromotive forces generated at the two junctions of the thermocouple are opposed to each other, consequently if the temperatures of the junctions are the same the forces will be balanced and no current will flow. However, if one temperature is higher than the other, there will be an excess of e.m.f. at the warmer junction, and the result will be a flow of current around the thermocouple which would cause a deflection in a galvanometer. The energy necessary to maintain this current is derived from the additional heat absorbed at the warmer junction.

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A current in the thermocouple, therefore, depends upon a difference in temperature being established between the two junctions. The "cold" junction is kept in a known and constant temperature as in a mixture of ice and water in a thermos bottle, and the "warm" junction is placed in contact with the material of unknown temperature. Since one temperature is known and since the e.m.f. generated varies directly with the temperature difference, the unknown temperature can readily be determined.

THE PRINCIPLE OF THE POTENTIOMETER

In actual practice it is a simple matter to determine the unknown temperature quickly when the apparatus is set up. However, it is well to know why certain instruments have to be used. The e.m.f. generated in the thermocouple can be measured in two ways: by the galvanometer and the potentiometer methods. The former measures the current directly and is the simpler one. However, it involves certain errors due to the resistance of the galvanometer in the circuit and also of the leads which are the copper wires connecting the junctions with the accessory instruments. This resistance varies with the length and diameter of the leads and especially with differences in temperature. True, it is possible to counteract this by introducing into the system an equal and opposite resistance; but the conditions causing variations in the resistance of the circuit have to be known and carefully standardized to obtain comparable results at different times.

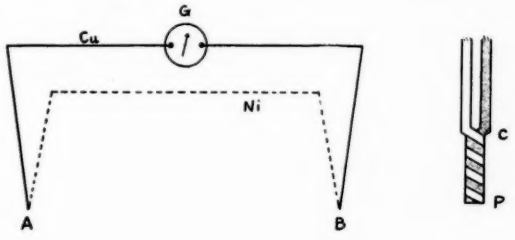
Fortunately these resistances may be disregarded by the use of a potentiometer. This is an ingenious device by which only the e.m.f. of the circuit, and not the current, is measured. No current flows at the time the measurement is made; consequently resistance ceases to be a factor, and the leads may be of any length and diameter and may pass through any variations of temperature without affecting the accuracy of the readings.

When a temperature is to be read, the e.m.f. of the thermocouple circuit is simply opposed by balancing against it a difference of potential supplied through a secondary circuit. When the two circuits are completely opposed there can, of course, be no flow of current in the system, and the galvanometer will stand at zero. The reading of the potentiometer is taken at that time and will show the magnitude of the e.m.f. drawn from the battery in the secondary circuit to balance that of the thermocouple. The reading of the potentiometer is divided by a value (obtained by calibration as described later) and the result will indicate the unknown temperature.

CONSTRUCTION OF THE SIMPLE THERMOCOUPLE

Any of the various types of thermocouples required by the biologist can easily be made in the laboratory. The two metals recommended are copper and constantan. The latter is an alloy of 60 per cent copper and 40 per cent nickel. Wire of these two metals may be purchased in a number of gauges and with silk, cotton or enamel insulation. Wire of small diameter such as B. & S. No. 34 gauge is recommended, and double silk covered insulation is generally preferable. It is advisable to coat the wire with shellac before using, to strengthen the insulation and to permit easy removal at the points without fraying.

A length of constantan wire, equal to the distance between the material of unknown temperature and the location of the thermos bottle, is taken; also a length of copper wire about 2 feet longer. The insulation is very carefully removed with a sharp knife from both ends of the constantan and from one end of the copper wire, the precaution being taken not to scrape the metal unduly with the knife. The copper is connected to one end of the constantan by winding the ends together to form a spiral as in Fig. 1-b. This junction is next evenly soldered. A good method is to melt a little solder upon a clean surface of a soldering iron and, after applying the flux, to immerse the junction in the hot metal from *C* to *P*, Fig. 1-b. This is to be the warm junction.



1-a 1-b

Fig. 1-a.—A thermocouple circuit. 1-b. A thermoelectric junction, enlarged.

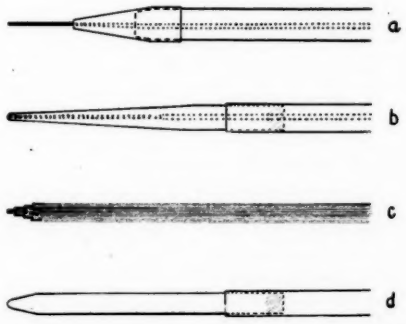


Fig. 2.—Different methods of finishing the thermocouple and thermopile junctions, enlarged.

The other end of the constantan wire is soldered in a similar way to a piece of copper wire about 2 or 3 feet long, to form the cold junction.

To prevent tangles of the wire and wear upon the insulation, it is well to enclose the body of the couple in a light rubber tube, leaving the junctions exposed. If instantaneous readings of temperature are required, it will be sufficient to coat the warm junction end with a protective surface such as a waterproof varnish or a lacquer, and fasten the rubber tube down near the end with adhesive tape, as in Fig. 2-a. If a momentary lag is permissible, better results will probably be obtained by enclosing the warm junction in a very small, thin-walled glass tube with one end sealed, as shown in Fig. 2-b. The junction is first dipped in melted paraffin and, while the wax is still soft, is pressed to the extreme tip of the tapered tube. This will exclude air and greatly reduce lag. Warming the tube will assist in getting the junction to

the tip. The open end of the glass tube is plugged to hold the couple in place. The rubber tube overlaps the glass slightly and is held in place with adhesive tape. Suitable glass tubing may be made by drawing out a test tube over a gas flame.

It is important to make the junction *CP*, Fig. 1-b, as short as is consistent with strength, probably about 2 mm. in length. Also when making a determination the junction should always be inserted into the material beyond the point *C*, preferably to a uniform depth of immersion.

The cold junction, which is to be placed in a thermos bottle, should be enclosed in a length of glass tubing sealed at one end, and allowed to rest in the ice water 2 or 3 inches. A convenient exit for the lead wires is between the glass and rubber tubing at the cold junction end. These wires are the two which connect the couple to the potentiometer, shown in Fig. 3.

SINGLE AND MULTIPLE THERMOCOUPLES

For the determination of one unknown temperature the arrangement shown in Fig. 3 will probably be satisfactory. "*A*" is the warm junction and

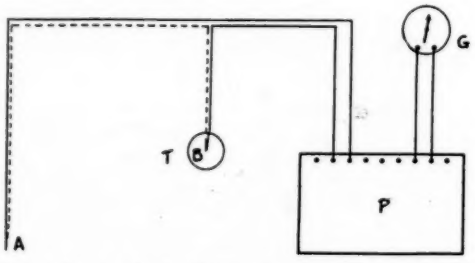


Fig. 3.—A set-up for a single thermocouple and also for a thermopile.

is to be placed in contact with the material of unknown temperature; "*B*" is the cold junction in the thermos bottle "*T*." The copper wires leading from the 2 junctions are connected to the potentiometer "*P*." The galvanometer "*G*" indicates when balanced conditions have been obtained. The distance from *A* to *B* may be any length required. The constantan wire in all the diagrams is represented by a broken line and the copper by a full line.

Occasionally it is necessary to determine 2 or more temperatures in quick succession or without removing the points. It is quite feasible to do this and to take readings over an extended period, with only a slight modification of the couple. A multiple couple is used for this purpose, and one type is shown in Fig. 4. It may have as many junctions as desired, each one being connected to the main constantan line by a branch of the same metal. The branches may be soldered to the main constantan wire at any convenient place. A detail drawing of the constantan connections is shown in the diagram "*C*" in Fig. 4. Each junction is then numbered and a full length of copper wire is run to a similarly numbered post on the selector switch "*S*."

Maintenance of the cold junction temperature and the method of calibrating the couple are described later in the paper.

THE THERMOPILE OR COMPOUND THERMOCOUPLE

Readings of temperature within 0.01° or less is sometimes necessary. It is seldom practicable to attempt to read this closely with the single couple, especially if used in connection with a galvanometer of ordinary low sensitivity. Large deflections in the galvanometer with small temperature changes may be obtained with a thermopile. This device is an adaptation of the principle of the thermocouple, and a diagram of its arrangement is shown in Fig. 5. It consists of a number of single couples in series. Each copper-constantan couple will develop between 37 and 40 microvolts per degree difference in temperature of the 2 junctions. When a number of couples are placed in series an additive effect is produced, and the total e.m.f. developed in the thermopile system may be comparatively large depending upon the number of couples used.

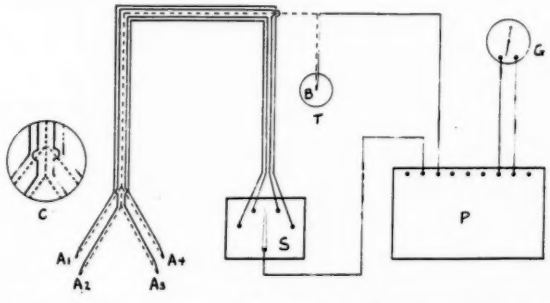


Fig. 4.—Arrangement for a multiple thermocouple.

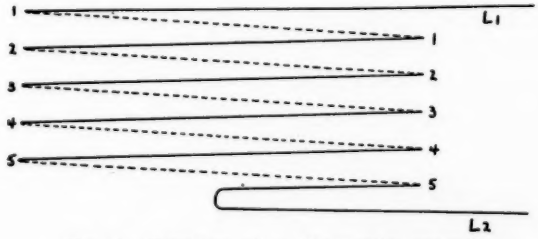


Fig. 5.—Diagram of a thermopile system.

CONSTRUCTION OF THE THERMOPILE

A board about 3 inches greater than the required length of the thermopile is obtained, and close to each end a row of short nails is driven in about one-quarter inch apart. There should be 2 nails for each junction plus an extra one at each end for the second lead wire. That is, for a thermopile of, say, 15 junctions 31 nails would be used at each end.

Wire of small diameter such as B. & S. Nos. 34 or 36 gauge is recommended, and double silk covered insulation is very satisfactory, although enameled wire may have an advantage where the diameter of the bundle is to be reduced as much as possible. Each odd-numbered nail at one end of the board is connected to the corresponding nail at the other end with a length of copper wire; and each even-numbered nail is connected in a similar man-

ner with constantan. All the wires are next given a coat of shellac to strengthen the insulation and to permit its removal at the tips without fraying. Adequate insulation is important and at this stage much trouble may be prevented.

When the shellac is dry, the wires are fastened down to the board about 6 inches from each end with a strip of adhesive tape. Then with a pair of scissors the wires are cut from the nails.

There is now a number of pairs of copper and constantan wires of equal length lying parallel in preparation for soldering. With a sharp knife the insulation is very carefully removed to expose about one-half inch of metal at each end. Emphasis must be placed upon the need of doing this with caution to avoid scraping the metal unduly. Each pair of wires is next connected by twisting to form a spiral as in Fig. 1-b. The wires must be so joined that a continuous connection is formed with all the junctions in series as shown in Fig. 5. The junctions are then soldered, and an electric soldering-iron is preferable as it remains hot while in use. The iron should have a flat surface, and upon this some solder is melted. After fluxing, each junction is immersed for an instant in the hot metal which should be deposited in a thin, even covering upon the wires.

The points are next cut back to a uniform length of about 2 mm. At this stage it is advisable to test each point to be sure that it constitutes a thermoelectric junction. This is done by connecting to a galvanometer the 2 lead wires marked L_1 and L_2 in Fig. 5, and touching each junction in slow succession with the warm fingers. A deflection indicates that the junction is in effective working condition. The tests should be made in a room free from sudden changes in temperature, otherwise there may be difficulties in controlling deflections due to variations in temperature of the 2 ends of the thermopile. A good plan is to make up each end of the thermopile into a temporary bundle but bending each junction away from the others so that none are touching. Then one bundle is placed in a wide test tube while trying out the other. Precaution should be taken when testing to avoid breathing upon the junctions.

The next step is one that requires especial care, namely, the complete insulating of the exposed metal of each junction. Unless this is done adequately the thermopile will not be satisfactory. For this purpose collodion has been found to give excellent results. Each junction is dipped into collodion beyond the exposed metal and allowed to dry thoroughly. Three coats are advisable to ensure a complete covering.

After thorough drying for several hours, the junctions at each end are made up into a permanent bundle. The points should each have an outside position. This can be done by placing 2 or 3 points at the tip and arranging the others closely together around the sides as in Fig. 2-c.

Several methods of finishing the thermopile are available. It may be enclosed in a glass tube, which has some advantages for use in physical chemistry but is too rigid for many purposes in biology. A satisfactory enclosure can be made with rubber tubing. This does not interfere with flexibility and

gives sufficient protection from injury and short circuits. Thin-walled glass tubing sealed at one end may be used at the warm end, as shown in Fig. 2-d. The bundle of junctions is dipped in melted paraffin and pushed to the tip of the tube as described for the simple thermocouple. The bore of the tube should be as small as possible. If instantaneous readings are essential, the warm junctions should not be enclosed in glass but be dipped 3 or 4 times in shellac or other suitable material to protect the collodion. This outer covering must not, of course, be soluble in ether otherwise the insulation would be ruined.

The connection of the thermopile with the potentiometer is similar to that of the simple thermocouple and is shown in Fig. 3.

MAINTENANCE OF THE "COLD JUNCTION" TEMPERATURE

The temperature indicated by the thermocouple is, as explained in the section on principles, the difference between the temperatures of the 2 opposite junctions, such as between *A* and *B*, Fig. 3. One of these temperatures,

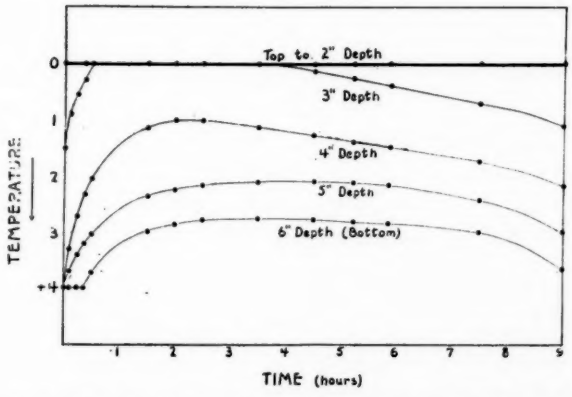


Fig. 6.—Showing fluctuations in temperature in a thermos bottle when not well filled with ice.

that at *B*, is required to be known; and the dependability of the couple as a precision instrument can be no greater than the accuracy with which the cold junction temperature is known. The simplest way to satisfy this requirement is to maintain the temperature uniformly constant.

Fortunately a constant temperature can readily be obtained in a suitable mixture of ice and water in a thermos bottle. The bottle should be made up with as much chipped ice from distilled water as it will hold and then be filled up with cold distilled water. Such a mixture will maintain for hours a temperature in the upper part of the bottle which will not fluctuate from zero more than 0.001 to 0.002°. This uniformity cannot be depended upon unless the thermos bottle is well filled with chipped ice. A bottle half-filled with ice and the remainder with water would produce a temperature gradient as in Fig. 6. This chart shows that the required temperature of zero is obtained only in the upper 2 to 3 inches, below which the temperature is continuously changing. A cold junction resting below the 3 inch level would cause large errors in readings.

When determinations are made over long periods it is advisable to insert a precision or a Beckmann thermometer in the cork at the cold junction end, and to note when the temperature begins to rise. Both thermometer and junction should, of course, rest at the same level in the ice water.

CALIBRATION

The number of microvolts which the thermocouple or the thermopile will generate per degree of temperature difference between the two junctions, now remains to be determined. This value is obtained through the potentiometer. The couple or pile is connected to the potentiometer and the cold junction placed in the thermos bottle. The warm junction is then exposed successively to 3 or 4 known temperatures. The number of microvolts generated at each temperature is divided by the number of degrees of temperature between the cold and warm junctions. The results should be practically a constant for all temperatures with which biologists are concerned, provided determinations closer than 0.01° are not required. If finer readings are necessary, certain precautions to be mentioned later have to be taken.

The accuracy with which the temperatures used in calibration are known will depend upon the precision required in the thermocouple. If an accuracy in reading within, say, one-tenth of a degree or less is satisfactory, the couple or pile may be calibrated by the use of water-baths, the water being stirred and the thermometer accurate to that extent.

For greater precision, fixed points in thermometry such as melting and transition points are commonly used. The following are suggested:

1. Transition point of manganous chloride: 58.09° C.
2. Transition point of sodium sulphate: 32.38
3. Freezing point of distilled water: 0

Transition points accurate to 0.01° may readily be obtained. For instance, with sodium sulphate, about 25 grams of the anhydrous form, c.p., are placed in a clean, wide test tube and moistened with distilled water. The temperature of the mass will then slowly rise to 32.38°. To prevent loss of heat when the water is added, the tube should be insulated and corked. The warm junction is pushed into the mass, and when the temperature ceases to rise and remains constant for a time the number of microvolts generated, as read on the potentiometer, should be noted. That value divided by 32.38 will give the constant sought.

In all subsequent work, for assurance that the couple or pile and its accessory apparatus are in reliable working condition, a frequent check-up with a known temperature is recommended.

HOW TO APPLY THE CONSTANT OBTAINED BY CALIBRATION

Having determined the number of microvolts generated per degree of temperature difference, and having established it as a constant C, it is then possible to read any unknown temperature to approximately 0.01°. This is done, of course, by dividing the number of microvolts generated by the con-

stant. The value for C should be reliable through the range of temperatures used in calibration and probably for all temperatures in which biologists are interested.

PRECAUTIONS NECESSARY IF READINGS ACCURATE TO 0.001° ARE REQUIRED

When temperatures are to be read with this accuracy especial care is necessary in both the construction and the use of the thermopile; and unless precautions are taken it is doubtful if the readings can be considered reliable.

1. The wire used sometimes has mechanical or electrical flaws, and must be checked for "homogeneity" or uniformity. This can be done in the laboratory by the method suggested by White[1] or wire already checked may be purchased from some of the dealers who supply wire for thermocouples.

2. The maintenance of the "cold junction" temperature previously described is obviously of increased importance in precision work; for the accuracy of determinations can be no greater than the constancy of the known temperature.

3. The temperature-e.m.f. relationship no longer takes the form of a straight line when measurements as close as 0.001° are to be made. Therefore the factor which, in determinations to 0.01° may be regarded as a constant, now increases slightly in value with the temperature. This departure from the linear relationship, however, may be adjusted by reference to the data given in the International Critical Tables, Vol. 1, pages 57, 58.

4. The transition points used in calibration should be determined to the third decimal place. The two points previously suggested would therefor be:

Manganous chloride,	58.089° C.
Sodium sulphate,	32.384

The transition points correct to the third place can be obtained only with material of exceptional purity such as after two or three recrystallizations. These and other transition points are given by Richards and Yngve.[2] The International Critical Tables, Vol. 4, page 6, list a number of transition points.

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2. Richards, T. W., and Victor Yngve: The Transition Temperatures of Strontium Chloride and Strontium Bromide as Fixed Points in Thermometry, J. Am. Chem. Soc. **40**: 89-95, 1918.

A SIMPLE APPARATUS FOR CLEANING COVERSLIPS*

By Harold Gordon, M.D., Ann Arbor, Mich.

THOROUGH cleaning of coverslips is an important detail of the duties of the technician in laboratories of histology, pathology, and bacteriology; but as usually conducted by the manual method, it is a time-consuming and irksome task. The following apparatus has been designed to facilitate this work and can be set up readily wherever compressed air is available. Several weeks' trial in the Pathological Laboratories of the University of Michigan has demonstrated its advantages over the old method of cleaning coverslips by hand. Breakages are less frequent and several packages can be cleaned at one time. The minimum of handling further insures freedom from grease, an important factor in insuring firm adhesion of the sections where the albumin fixative method is employed. The superiority of the new method over the old, in maintaining adhesion between section and coverslip has been demonstrated by actual experiment.

APPARATUS

A glass cylinder open at both ends, 2 feet in length and 2½ inches in diameter, is fitted at one end into a Buchner funnel of somewhat larger capacity. The cylinder is firmly sealed into the funnel with a paste of litharge and glycerin. After allowing the paste to dry, the union is permanent and leak proof. The cylinder is attached to a suitable metal stand and the tapering end of the funnel is connected to an air pipe by means of stout rubber tubing. The upper end of the cylinder is fitted with a rubber cork, pierced to allow the insertion of two glass tubes, each about 10 mm. in diameter. Experience has demonstrated the advantage of a double outlet in preventing the cork stopper from being displaced when the air current is turned on. Both tubes are bent in "U" fashion, with one limb of the "U" short, the other long (see Figs. 1 and 2).

METHOD

The cylinder is filled to about a quarter of its capacity with soap solution and the coverslips are placed in the solution. (The soap solution has been found to be superior to any of the usual acid cleaning fluids.) The rubber cork is replaced and the air current turned on. Four half-ounce packages of ⅞" coverslips (or more of the smaller size) and three packages of the larger sizes have been found to clean quite readily. The air is allowed to bubble through the soap solution, continuously agitating the cover glasses, for about ten minutes and then shut off. The coverslips are next rinsed in four changes of distilled water; each change being aërated for a few minutes to insure re-

*From the Department of Pathology, University of Michigan.

moval of every trace of soap and complete the separation of the coverslips. The water is next drained off and replaced with 96 per cent alcohol and air is again allowed to bubble through for five to ten minutes. The alcohol should be saved and may be used repeatedly. The coverslips are then transferred to a metal tray covered with paper towelling. The tray is placed on top of an incubator or oven, and the coverslips are allowed to dry in a dust-free atmosphere. It may be well to interpolate a warning at this point. The use of a wooden rather than a metal tray or dish is attended by some risk of fire, as also is the insertion of the alcohol moistened coverslips inside

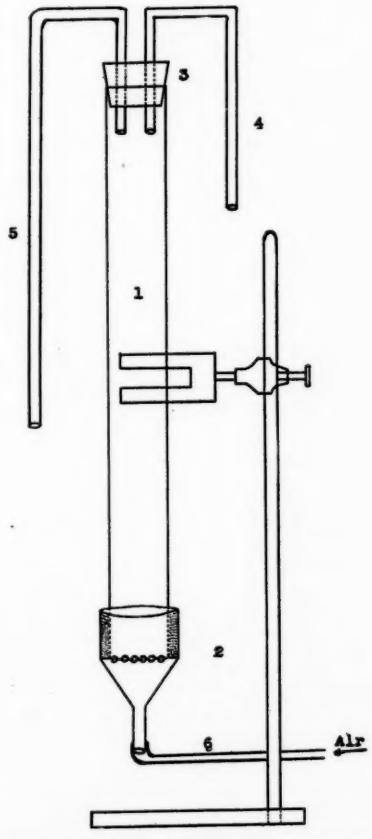

Fig. 1.—*1*, Glass cylinder for receiving coverslips. *2*, Buchner funnel. *3*, Perforated cork for exit tubes, *4* and *5*. (These can conveniently be twisted around to empty into a sink or basin.) *6*, Rubber tubing connecting with compressed air line.

instead of on top of the incubator or oven. The actual washing of the coverslips can easily be accomplished in half an hour and while they take some time to dry, the interval may be utilized at other tasks since they require no further attention. Although simple in construction and principle, for the successful operation of the apparatus as outlined above certain precautions must be borne in mind. The soap solution should not have such a high content of soap as to render the solution too "bubbly" while aërated. A little experience will soon enable the operator to gauge the correct proportion of soap to use. An excess of soap causes the bubbles to escape by the

exit tubes, the solution as a result either "boiling dry" or overflowing in a messy manner. The long ends of the exit tubes should be so arranged as to reach into a sink or a large basin. The air should not be allowed to enter too suddenly or forcibly and the exit should be sufficiently wide to make for a ready escape of the air. The double exit tubes are of special advantage where the water supply is free of hardness or chemicals since the rinsing

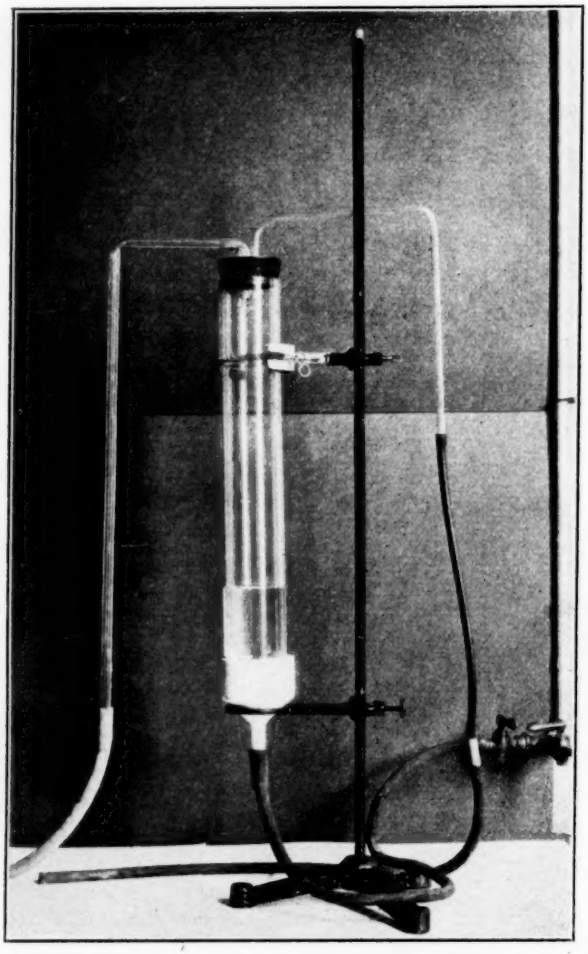

Fig. 2.—Actual photograph of apparatus. The two lines within the cylinder are due to reflections from its wall. The exit tubes end immediately beneath the perforated stopper at the top.

process may then very readily be effected by connecting one exit tube to the water tap and disconnecting the air-tube to allow the water to run in from above and escape from below, the other exit tube allowing the air between these two channels to gain exit, thereby not disturbing the rubber cork. Finally, the rinsing must be thorough. The least trace of soap in the alcohol spoils it for subsequent use and causes the coverslips to be streaked. Complete removal of the soap insures beautifully clear and glossy glasses.

A NEW METHOD FOR STAINING BACTERIUM TULARENSE IN TISSUE SECTIONS*

By Lee Foshay, M.D., Cincinnati, Ohio

DURING the course of some work on experimental tularemia in animals I have found that several dyes are capable of staining the Bacterium tularense in tissue sections in addition to Twort's light-green-neutral-red mixture already used and reported by Ledingham.[1] A well differentiated Giemsa stain is unquestionably the best of all but there are other stains almost as good which are simpler of management and quite adequate for routine work. Of these stains the best, in my experience, is nile blue sulphate. When the bacteria are present in large numbers and arranged in fairly large masses in the tissues, as happens frequently in the spleens and lymphoid tissues of rodents and also occasionally in man, the dye may be used alone with good results. For routine work on animal tissues this is an adequate and rapid stain. The method is simple.

Fig. 1.—Photomicrograph of guinea pig spleen showing one large group and several smaller groups of B. tularense lying free in the intercellular spaces. Nile blue sulphate stain. Magnification 1550 Diam.

Deparaffinate the sections as usual and carry them through the alcohols to water. If heavy metal fixatives have been used, remove the metallic salts in the usual way. Stain in a saturated aqueous solution of nile blue sulphate for from five to six minutes. Wash rapidly in water, dehydrate rapidly through the lower alcohols to absolute, then carry through the xylols and mount as usual.

*From the Christ Hospital Institute for Medical Research.

When the bacteria are present in smaller groups they are somewhat difficult to find because of the relatively low contrast between the stained bacterial bodies and the cellular background. By diluting the stain and adding sufficient safranine to produce a purplish blue color, and using this mixture for overnight staining, a much higher color contrast is obtained. This renders the bacteria more readily visible and much easier to photograph. The method follows:

To 60 c.c. of distilled water add 10 c.c. of aqueous saturated nile blue sulphate and 6 c.c. of 1 per cent aqueous safranine. When the deparaffinated sections have reached the water stage, stand them in this solution to stain overnight. I have obtained good stains of certain tissues after only two hours in this solution but sixteen hours is better, although an hour or two more or less seems to make no appreciable difference. After staining wash rapidly in water and pass through the alcohols and xylols as usual.

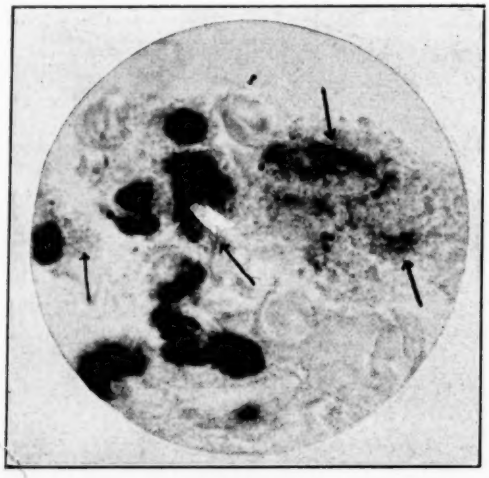

Fig. 2.—Photomicrograph of section of human lung (case of Bunker and Smith) showing both intracellular and extracellular groups of B. tularense. Unstained sections from this case were very kindly supplied by Dr. G. W. McCoy, Director, National Institute of Health. Magnification 1550 Diam.

This method has been used to demonstrate the Bacterium tularense in the lymph glands, spleen, liver, lung, kidney, and skin of the guinea pig; in the lymph glands, lung, and spleen of the rabbit; in the lymph glands, liver, spleen, lung, and kidney of the mouse; and in the lung from a fatal human case. This fatal case with necropsy findings is being reported in detail elsewhere. So far as I am aware, it is the first time this organism has been demonstrated in human tissues.

The choice of a fixative solution for the fresh tissues seems to be a matter of no importance. When the bacteria are present they are well demonstrated by the nile blue sulphate in tissues fixed in Zenker's, Orth's and Dominici's solutions, ethyl and methyl alcohols, and formaldehyde. There seems to be no difference in the tinctorial qualities following the use of any of these. When examining the mounted sections the use of an orange filter aids in giving added depth of color to the bacteria, thus increasing the con-

trast. As a matter of passing interest this stain also reveals the presence of a wide variety of bacteria in tissues. Pneumococci and streptococci are well shown in pneumonic lungs and in the smaller animals the typhimurium, bronchisepticus and lepisepticus are often encountered in various organs.

The color imparted to the bacteria is not a particularly good one for photographing. The addition of safranine produces a better shade for this purpose than does the nile blue sulphate alone. A number of unsuccessful attempts have been made to improve on this.

A word is due regarding the finding of B. tularense in the guinea pig liver. I believe they have never been seen in this organ before. When normal guinea pigs are infected from cultures or from the spleens of animals dead of tularemia the survival period after infection is usually six days and four days respectively. Repeated examinations of the livers of such animals have consistently failed to reveal any bacteria either in or around the characteristic focal necroses. I have tried Giemsa stain, Twort's light-green-neutral-red mixture as recommended by Ledingham, light-green followed by safranine, and nile blue sulphate both alone and in combination with a number of other dyes. Bacteria are easily demonstrable in the spleens and in other organs but never in the liver. This was also Ledingham's experience.

The only guinea pig liver which has revealed the organisms in the focal necroses is the one from an animal that lived eleven and one-half days after infection from a culture. An effort had been made to immunize this animal by intraperitoneal injection of human convalescent serum. This did not protect but it did prolong life far beyond that of the other animals of the series and of the control animal.

SUMMARY

A method is presented for staining the Bacterium tularense in tissue sections. This method has revealed the organisms in human lesions from two different fatal cases, and has demonstrated the organisms in the liver of one guinea pig.

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NONPOLARIZABLE ELECTRODES*†

By John W. Williams and Walter C. Bosch, New Orleans, La.

A NONPOLARIZABLE electrode seems to some an impossibility. Nevertheless electrodes can be made which polarize less than those at present on the market. We have devised such an electrode which because of less heat production can be utilized in growing bacteria at more near their nor-

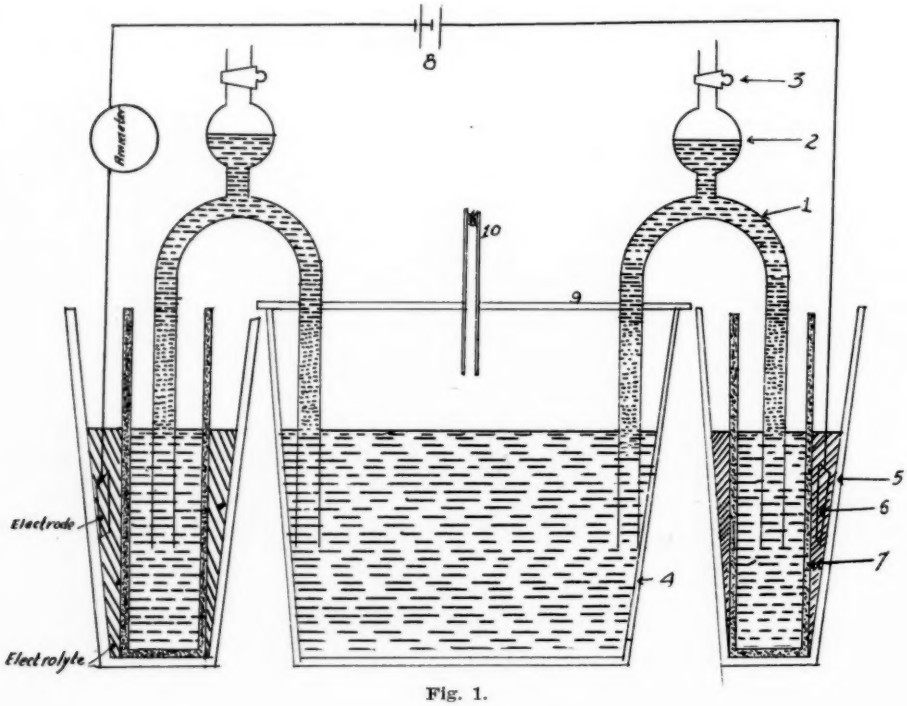

Fig. 1.

mal environs. By this means it is hoped an additional factor may be contributed to the classification of bacteria (i.e., a growth positive or growth negative organism) and that the lethal and optimum current for bacterial growth may be determined. In addition to the application of this device to bacteria it may be used in noting activities of inert particles. The media in which the bacteria must be grown are liquid since the electric current will disrupt the solid media and cause a short in the circuit.

Description of Device. Cross section. The device consists of a jar (4) in which the bacteria are to be incubated. This jar protected by a cover (9)

*From the Departments of Pathology and Physics, Tulane University.
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†Aided by a grant from the Schwartz Research Fund.

into the top of which a tube (10) projects. This tube is plugged with cotton and is for the purpose of injecting bacteria into the media. Like tubes may be placed adjacent to each electrode in order that bacteria may be drawn off from the poles whenever desirable. We note by the plate that ends of two electrodes project into this jar while similar ends of the same electrodes are received in porous cups (7). It will be noted by the line shading that the media in the electrodes, the jar and the described cups is the same, i.e., a liquid media. On the top of each electrode will be noted a bulb (2) half-filled with the liquid media. This media is capped by a negative pressure chamber resultant from the closed stopcocks (3). The procedure in effecting this fill is to open the stopcocks and fill the electrodes with the media from (4) and (7) by means of negative pressure and then close the stopcock. In this way should any fluid evaporate from the electric conduction system it would be replaced from (2) and there would be no interruption of current. The porous cups rest in a jar (5) filled with electrolyte in which electrolyte is the electrode (6) connected to direct current at (8).

This apparatus can be first filled and then sterilized. The porous cup may also be covered and in this way a further possibility of contamination avoided. The porous cup is so constructed that the electrolyte does not diffuse into it and contaminate the media. Care must be taken that the height of the electrolyte and media be kept the same, and that both exert almost equal osmotic pressure. Although there will be slight change in the media in the porous cup as a result of fluid diffusion, however, sufficient diffusion will not take place to markedly influence an organismal growth so far removed as in this case. In the herein contained diagram the fluid levels are too high. It is far preferable that they be just above the electrodes.

A DIAGNOSTIC AID IN OBSCURE HEMATOLOGIC CONDITIONS*

By Carl Reich, A.B., M.D.,† New York, N. Y.

THE hematologist is sometimes confronted with the difficulty of making a diagnosis in cases which appear to be in aleucemic phases of leucemia, but do not show sufficient numbers of pathologic cells in the stained films to permit a definite opinion. These patients usually present the picture of a severe anemia with leucopenia, and it is necessary to either await further changes in the peripheral blood picture or do a bone marrow biopsy.

The following method of procedure has shown itself to be of value and may at times obviate the necessity of a biopsy of the bone marrow. Ten c.c. of blood are withdrawn from the vein in a syringe and mixed with 2 c.c. of a 1.4 per cent sodium oxalate solution. This has been shown to be an isotonic anticoagulant which does not change the volume of the cells.‡ Heparin and hirudin may be used, but they are expensive and often not available. This mixture of oxalated blood is then centrifuged in an ordinary small centrifuge tube for about fifteen minutes. The supernatant plasma is removed and discarded, using a capillary pipette with a rubber nipple attached. By carefully manipulating the same pipette the buffy coat containing the white blood cells can be sucked up and used to prepare smears on cover slips or slides. These are then allowed to dry and stained and examined in the usual way.

The method outlined above gives us a histologic picture of the white blood cell content of 10 c.c. of blood, and will often clinch a diagnosis which could not be confirmed from films prepared from the usual single drop of blood.

*From the Achelis Laboratory, Lenox Hill Hospital, New York.
Received for publication, May 21, 1931.

†Assistant Bacteriologist and Attending Hematology Clinic, Lenox Hill Hospital, New York.

‡Haden, R. L., Technique of Determination of Relative Mass, Individual Cell Volume, and Volume Index of Erythrocytes of Man. J. Lab. & Clin. Med. 15:736, 1930.

AN IMPROVED DISTILLING COLUMN*

By Charles B. DeWitt, Memphis, Tenn.

THE distilling column shown in Fig. 1 was designed for close fractionation of small quantities of liquids having relatively high boiling points. It has been found satisfactory for separation of lower boiling liquids as well. It combines high efficiency with unusual strength and compactness, and gives the operator control over the distillation to a degree that is unmatched by any other column the writer has used.

The device will be readily recognized as an adaptation of the West condenser† in combination with a Vigreux column. The inner tube is made from light or medium wall Pyrex tubing whose diameter is dependent on the length

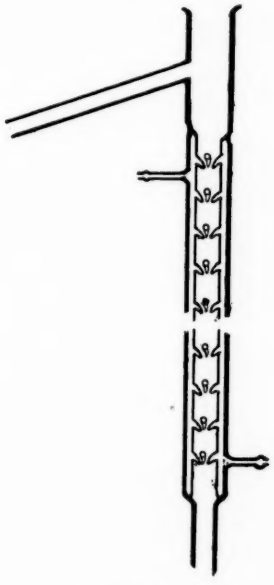

Fig. 1.

of the column and the quantity of material to be used with it. An internal diameter of eight millimeters is sufficient for handling small quantities. The indentations are made by heating small spots in a sharp flame and pushing them in with a bent knitting needle. A deep file mark on the latter serves as a depth gauge. The indentations are made in groups of four to eight around the tube, and the groups are spaced about three-fourths of an inch apart. This tube must be well annealed, particularly if the column is to be used for low pressure distillations.

*From the University of Tennessee.
Received for publication, May 11, 1931.
†West, E. S.: Industrial and Engineering Chemistry 20: No. 7, p. 737.

The jacket is made of heavy wall tubing of a diameter that will allow a space of about one millimeter between it and the inner tube. Other construction details are clearly shown in the drawing. For the smaller sizes, the reducer shown at the lower end is unnecessary, as the jacket tube is small enough to go into the neck of a flask.

This column has no thin-walled bulbs or fragile siphon tubes that are easily broken in handling. The jacket, in addition to giving the column great strength, affords protection against air currents that often momentarily stop the output of distillate from other columns and cause exasperating fluctuations in temperature. To secure the necessary condensation, a stream of compressed air is led into the jacket through the upper side tube. This puts the heart transfer from the inner tube under control of the operator, and materially speeds up distillation. If a column of suitable size is used, it is seldom necessary to interrupt distillation to drain liquid from the column.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PNEUMOCOCCUS TYPING: A Microscopic Method by the Use of Stained Organisms, Calder, R. M. J. A. M. A. **97:** 698, 1931.

The organisms are prepared in suspension as for an ordinary macroscopic typing, and a drop is taken up by a capillary pipette and transferred to each of the four cover slips. Another capillary pipette is held for a moment in a Bunsen flame to seal its end and bend it into the form of a small hook. This hook is dipped into the gentian violet solution, and the adhering dye is allowed to dry completely. The stain is worked into the drop of bacterial suspension on the cover slips and, as the film of dye on the hook is very thin, the amount mixed with the drop of suspension can be controlled accurately. The amount of dye to be worked into the drop to secure the best staining can be gaged easily after a little experience. If the dye as it comes off the hook spreads unevenly through the drop, a second capillary hook, without stain, can be used to secure an even mixture. The organisms take up the dye selectively and appear as deeply stained bacteria in an unstained or faintly bluish medium.

After the drop has been stained, equal volumes of the diluted serums are added by capillary tube and thoroughly mixed with the suspension by means of capillary hooks, care being taken, of course, to use separate stirring rods for each of the various preparations so as not to mix the serums. In the ordinary typing, four hanging drop preparations are set up, one for each of the three fixed types and one without serum for control. The cover slips are inverted over hollow-ground slides, sealed and placed in the shaking machine, where five minutes of shaking usually bring about sufficient agglutination to be read with the 4 mm. objective.

ANEMIA: Pernicious, Relation of Achlorhydria to, Moschcowitz, E. Arch. Int. Med. **48:** 171, 1931.

From a fairly comprehensive review of the subject the author concludes that:

1. Achlorhydria is such a constant sign in pernicious anemia that probably no case is valid unless achlorhydria is present.

2. Achlorhydria is not the result of the disease but is primary. Evidence for this is shown in the fact that there is no diminution of hydrochloric acid in the progress of a case of pernicious anemia and that achlorhydria is present from the onset, that achlorhydria persists in the stage of remission and that it has been found frequently for years before pernicious anemia became manifest.

3. Achlorhydria occurs normally in a small percentage of persons. In order to determine this percentage, it is essential that mass studies be made with not only the fractional method of testing gastric secretion, but the tests with neutral red and histamine as well. The percentage of persons with achlorhydria apparently increases with each decade. It is extremely rare in childhood.

4. Achlorhydria is often present in certain families. In families in which a case of pernicious anemia has occurred, the incidence of achlorhydria is much higher than normal. As this incidence increases in each decade and as achlorhydria is exceptional in childhood, achlorhydria itself is not transmitted but only the tendency thereto.

5. Pernicious anemia is frequently hereditary and familial. Whether pernicious anemia is always hereditary and whether it is a dominant or recessive character and transmissible according to the mendelian law cannot be determined until such families are studied with

particular reference to achlorhydria and with accurate hematologic examinations being made for three generations at least.

6. The available evidence is not convincing that Bothriocephalus latus causes pernicious anemia. There is ground for believing that such reported cases represent instances of true pernicious anemia that happen to be associated with infestation with Bothriocephalus.

7. There is a definite relation between achlorhydria and anemia, of the pernicious, secondary and chlorotic types. The blood of relatives of patients with pernicious anemia shows changes, which sometimes may be regarded as the earliest or preclinical evidence of the disease. For diagnostic purposes, it is important to recognize this preclinical phase.

8. The development of pernicious anemia after an acquired achlorhydria, for instance after complete gastric resection, has not definitely been proved.

9. Many of the so-called "severe anemias" of pregnancy (exclusive of those due to hemorrhage or sepsis) represent cases of true pernicious anemia in which the patients have become pregnant. Whether the pregnancy is the inciting factor in patients with a constitutional tendency remains to be proved.

10. The anemia of sprue is often associated with achlorhydria. It is not yet established how often achlorhydria is acquired or is a constitutional tendency in this disease.

11. Achlorhydria is the most tangible but not the only evidence of the constitutional background of pernicious anemia. In all probability pernicious anemia represents a combination of a deficiency disease and a lack of a gastric hormone.

TISSUE: Modification of Mallory-Heidenhain's Differential Staining Method and Adaptation to Formalin-Fixed Material, Kernohan, J. W. Am. J. Clin. Path. **1**: 399, 1931.

The formalin-fixed tissues are washed in running water or ammonia water for a short time. The latter procedure serves to prevent the accumulation of "formalin precipitate" on the stained preparation. The tissue is then placed for four days in Weigert's primary mordant for myelin sheaths (potassium bichromate 5 gm.; chromium fluoride 2 gm.; water 100 c.c.); and for two days in Weigert's secondary mordant for myelin sheaths (acetate of copper 5 gm.; chromium fluoride 2.5 gm.; acetic acid, 36 per cent 5 c.c.; water 100 c.c.; formalin 10 c.c.) It is then embedded in paraffin and stained in the usual way with Mallory's phosphotungstic acid hematoxylin or other staining methods requiring Zenker's fixation. This differentiates the neuroglia, fibroglia, etc., as satisfactorily and clearly as if the tissue had been fixed in Zenker's solution, and excellent results have been obtained after the tissue has been fixed several years in formalin.

VACCINE THERAPY: Principles of, Kolmer, J. A. Am. J. Clin. Path. **1**: 379, 1931.

1. Vaccines have proved of more value in the prevention than in the treatment of disease.

2. Methods of preparation of stock and autogenous vaccines appear to have an important bearing upon their therapeutic value.

3. Successful vaccine therapy demands accurate bacteriologic diagnosis, especially the employment of proper and acceptable methods for securing the important organisms of infection for the preparation of autogenous vaccines.

4. Well-prepared autogenous vaccines are to be preferred to stock vaccines in the treatment of disease.

5. Whenever possible vaccines should be composed of living organisms of reduced or modified virulence or so prepared as to approach this state as nearly as possible.

6. Bacteriologic toxins wtih or without modification should always be incorporated or used whenever possible in the preparation of vaccines.

7. The sterilization of vaccines with chemical agents or by filtration is to be preferred to sterilization by heat.

8. Prophylactic immunization is largely dependent upon specific antibody production whereas therapeutic immunization is probably due in part to both specific and nonspecific effects.

9. The intracutaneous injection of vaccines may be of more prophylactic and therapeutic value than subcutaneous injection.

10. The route of administration and dosage of vaccines have some bearing upon the results and should be selected according to the indications and requirements of individual cases.

11. Vaccine therapy is sometimes of value in the treatment of acute infections.

12. In the treatment of some chronic diseases vaccines have met with a measure of success and are worthy of further use especially by those possessing special skill and experience and with due regard for important technical details involving their preparation and administration.

TISSUE: A New Clearing and Mounting Fluid for Small Insects, Mukerji, S. Ind. J. Med. Res. **19:** 281, 1931.

The following formula is presented:

Chloral hydrate	0.5 gm.
Distilled water	1 c.c.
Glycerine	1 c.c.
Lactic acid (extra pure)	2 c.c.
Glacial acetic acid	2-4 minims
Formol	0.5 c.c.

The medium is easy to prepare and no filtering or heating is necessary since the fluids are perfectly miscible. When pure ingredients are used the resultant fluid has the advantage over lactophenol in that it is perfectly colorless and transparent. The ingredients should be mixed in the order given above and it is advisable to use fresh medium each time.

Method of Clearing: Some clearing medium is dropped with a pipette on the cell of an excavated slide and the entire object or its part as the case may be to be cleared is carefully dropped into the medium and the cell covered with a cover glass. Immediately the clearing of the parts begins to take place which should be watched through a binocular microscope. The medium may conveniently be used a permanent mountant with satisfactory results. A Nematode and a sandfly larva mounted in this medium more than a month back although sufficiently cleared have kept their original color.

URINE: The Number of Formed Elements in Heart Disease, Stewart, H. J. and Moore, N. S. J. Clin. Invest. **9:** 409, 1930.

The number of casts found in twelve hours is usually increased in patients suffering from cardiac disease, although the number may be normal. If the average numbers are considered, the greatest numbers were passed by those patients suffering from heart failure of the congestive type; the numbers were fewer after recovery and fewer still in those who had never suffered from this illness. Granular casts were frequently found.

The number of red blood cells in the urine of patients who had experienced cardiac decompensation was frequently greater than the highest normal value, but within the limits in those who had never suffered from heart failure. The average number of red blood cells found in those cases which had never experienced heart failure was twice as great as that in normal individuals; in those who were suffering from heart failure or had recovered from it, however, the average number was 10 to 15 times as great as in normal individuals.

The number of white blood cells was normal in the urine of those patients who had not suffered from heart failure but the average number was approximately twice the average observed in normal individuals. The number was usually within the normal range both during and after recovery from cardiac decompensation; the average number, however,

was greater approximately 9 and 3 times respectively than that in normal individuals, the average being less in patients without heart failure than in those who had recently recovered from it and less than in those who were still suffering.

LEPROSY: Histamine Test in the Early Diagnosis of, Rodreguez, J. and Plantilla, F. C. Phil. J. Sc. **46:** 123, 1931.

When a dilute solution of histamine is pricked into the normal skin, a reaction takes place in about twenty seconds, starting with the appearance of a circular, sharply defined, local reddening surrounding the prick, and measuring when fully developed from 3 to 4 mm. in diameter. This is followed in another fifteen to thirty seconds by a flush or "flare" that appears on the surrounding skin. It is of the utmost importance to distinguish this flare from the local red reaction. The flare is dark red or scarlet contrasting with the brighter shade of the latter; it has diffused and often crenated borders that may extend from 2 to 3 cm. from the center of the reaction. Soon after the appearance of the flare, a discrete wheal forms at the site of the prick; this is generally at its maximum development in from three to five minutes, at which time it measures from 3 to 4 mm. in diameter and about 1 to 2 mm. in height. The wheal usually occupies the area originally covered by the local red reaction, although in many cases the two do not coincide, the wheal being usually smaller than the localized red area.

The full reaction of the normal skin to histamine, consisting of the local redness or vasodilatation, the flare, and the edema or wheal has been called by Lewis the "triple response."

In most of the author's tests, they have used a 1 to 1,000 dilution of the phosphate in normal salt solution. With stronger solutions a larger flare is occasionally obtained, but the reactions are not as constant as with the 1 to 1,000 solution.

A small drop of the solution is carefully placed within the suspicious macule to be tested and another is dropped on normal skin at least 2.5 cm. from the border of the lesion for control. With a sharp pin, a prick is made through the drop into the skin underneath, taking care to exert just sufficient pressure to drive the point through the epidermis without causing any bleeding. The histamine solution is wiped off immediately, and the pricks are closely observed under good natural light.

The test is said to be negative when the complete response is elicited and positive when the flare is absent.

There are some individuals on whom the normal reaction is diminished; in a few, the flare is so faint as to be practically absent. When the response is weak and the skin tested is on an extremity, the flare may be brought out to its maximum extent and intensity by previously congesting the extremity with the help of a broad rubber band or the pneumatic cuff of a blood-pressure apparatus.

RESULT OF THE HISTAMINE TEST IN LEPROSY

In the pale macule: The flush is always absent in the depigmented macule of leprosy. When the histamine prick is made just outside the border, a flare develops on the normal skin but stops sharply at the border and does not extend into the macule. When the prick is made just inside the border, the flare is prevented from appearing even on the bordering normal skin.

A word of caution must be given at this point. The flare generally masks the local redness following the histamine test on the normal skin. When the flare is abolished as in a leprotic macule, the local redness becomes prominent and may be mistaken for the flare by the beginner. The area of local redness is sharply localized, circular in shape, bright red or pink in color, extending at the most 2 or 3 mm. beyond the wheal, and tends to become cyanotic before fading. On the other hand, the flare is not definitely localized, the size is usually about 3 to 4 cm. in diameter, irregular in shape, although it tends to be oblong with its long axis along the length of the member, and the color is dark red. On fading the flare becomes speckled, but the color remains the same from beginning to end.

The wheal in the macule is usually of the same size as that on the normal skin. Sometimes the edema may be less; at other times the wheal develops faster in the macule, reaching its full development in two minutes, while the wheal on the control skin is at its height in three to five minutes. The ultimate size, however, is almost the same.

In the reddish macule: When the redness of the lesion is marked, only the wheal may be elicited; but when the color is not so striking the local redness may be seen.

When hyperesthesia is present, as is usually the case when the lesion is bacteriologically positive, the flare is not constant. In a few macules the flare is present; in the majority of the cases it is absent. If there is accompanying infiltration or edema so marked that the skin looks tense, glistening, and bright red in color, the wheal is apt to be slight or absent.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

*Trauma, Disease, Compensation**

THE title is at once suggestive, reminding the reader to what a great extent compensation features enter today into the treatment of injury and disease among the working classes. We find a comprehensive volume which on the whole, however, is rather disappointing. The first chapter deals with the basis and scope of workmen's compensation and the discussion of the problem is very satisfactory, presenting as it does actual judgments rendered. From this point on, however, in the discussion of the various diseases which may or may not become compensable, the information given does not seem to be quite definite enough. This appears to be due to the fact that with some exceptions, the references recorded are not from original sources or from legal decisions but from very general textbooks and systems of medicine. One would be unable on the witness stand to quote with any feeling of authority most of the statements made but would feel the need of first consulting the original authority and making a more detailed study of the specific problem.

Furthermore there are several subjects which come into everyday medico-legal questions today which are scarcely touched upon. Such is the general problem of carbon monoxide poisoning.

The final chapter dealing with established ratings for permanent disability following accidents is helpful.

Unfortunately there are few authoritative volumes on this general subject and until more appear the present one will be of distinct aid. The reviewer hopes, however, that in his next edition the author will devote himself more to specific cases than to generalities.

Ideal Marriage. Its Physiology and Technique†

THIS is the type of volume that can be reviewed in several ways. Many will declare that there is no justification for a treatise on the technic of coitus and that the volume should be suppressed. Others will hail it as a pioneer in the emancipation of current thought from the prudery of the past. The truth lies somewhere between.

The reviewer has felt that the test of the book is not in its reading but in its ability to solve the marital problems which its author assures us that it will help solve. For this reason we have delayed publication of the review for several months after the reading of the volume during which time we have used it in actual practice in a small selected series of situations in which it appeared that such a frank exposition would be desirable. From this study the reviewer has reached the following conclusion:

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*Trauma, Disease, Compensation. A Handbook of Their Medico-Legal Relations. By A. J. Fraser, M.D., Chief Medical Officer, Workmen's Compensation Board, Winnipeg. Cloth. Pages 524. Philadelphia, F. A. Davis Company, 1930.

†Ideal Marriage. Its Physiology and Technique. By Th. H. Van de Velde, M.D., formerly director of the Gynaecological Clinic at Haarlem. Translated by Stella Browne. Introduction by J. Johnston Abraham, C.B.E., D.S.O., M.A., M.D. Cloth. Pages 323. New York, Covici-Friede, 1928.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

While the flavor of the volume is distinctly continental and the author gives to the art of intercourse a glory and importance which is scarcely bestowed upon it in America, there is at the same time no doubt that many of the neuroses, especially among women, are based upon sex and are often due to failure of sexual gratification. There is no doubt that much of this is due to ignorance on the part of the mate. The reviewer has been able to clear up such situations entirely by allowing both parties to study the book.

It is a volume which in the hands of the light minded could do much harm. It is one which should be read by all physicians who are seriously endeavoring to better the lot of their patients. It is one which can unhesitatingly be recommended to those who the physician feels are in need of the information contained therein and who are capable of intelligently interpreting the information.

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*Legal Medicine and Toxicology**

WHILE it is true that in medical specialization there has developed a group of specialties related to expert testimony such as the toxicologist and the alienist, it is equally true that the ordinary doctor finds himself called into court with increasing frequency. Sometimes he is called as an ordinary witness, often as an expert. It becomes increasingly desirable that the doctor have available for his instruction reference volumes on the doctor as an expert in court. Most of the problems which he will be called upon to face are treated in Webster's textbook.

Thus, in the first section, on legal medicine, subjects such as the following provide the physician with a wealth of information on the basis of which he is better equipped to conduct himself properly on the witness stand: "Legal Procedure, The Coroner's Inquest, The Right to Practice Medicine, Contractual Relations Between Physician and Patient, Privileged Communications, Dying Declarations, The Making of Wills, Obligations of the Physician to the State, Death Certificates and Birth Certificates, The Prescribing of Narcotics and Alcoholics, Malpractice, Criminal Malpractice, Identification of the Living and the Dead, Death in Its Medicolegal Relations, Impotence and Sterility, Rape, Legitimacy, Paternity, Abortion, and Mental Disorders in Their Medicolegal Relations." Subjects such as these come into the problems of every physician at one time or another.

The second section, devoted to Toxicology, represents a more highly specialized field, one in which the general practitioner need never qualify himself, but at the same time one in which the clinical pathologist should be well grounded.

Doctor Webster's position as professor in medical jurisprudence in Rush and his former association with Peterson and Haines in their volume on Legal Medicine and Toxicology insure to the reader an authoritative exposition of the subject.

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*Legal Medicine and Toxicology. By Ralph W. Webster, M.D., Ph.D., Clinical Professor of Medicine (Medical Jurisprudence) in Rush Medical College, University of Chicago; Professorial Lecturer in Medical Jurisprudence and Toxicology in the University of Chicago; Toxicologist to the Coroner's Office, Cook County, Illinois; Attending Chemist, Presbyterian Hospital, Chicago; Director of Chicago Laboratory, Clinical and Analytical. Illustrated. Pages 862. Cloth. Philadelphia and London, W. B. Saunders Company, 1930.

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EDITORIAL

The Effect of Carbon Monoxide Poisoning on the Heart

CARBON monoxide exerts its poisonous action by combining with the hemoglobin of the blood thereby depriving the blood of oxygen. The affinity of carbon monoxide for hemoglobin has been estimated as being about 250 times the affinity of oxygen for hemoglobin. Therefore, when once carbon monoxide has become attached to hemoglobin, oxygen is crowded out as it were, and, being deprived of its method of transportation through the blood to the tissues, it can no longer reach the latter. As a consequence the cells of the organs and tissues of the body are virtually asphyxiated, dying for lack of oxygen. The most striking anatomic change resultant on this consists in minute hemorrhages, blood passing out through the damaged blood vessel walls into the tissues. This may occur in any tissue of the body, but since the nerve cells of the central nervous system are the most sensitive to oxygen deprivation, the most characteristic and constant findings in carbon monoxide poisoning are in the brain. At autopsy small hemorrhages or extravasations are found scattered through the brain substance. If the victim lives long enough these hemorrhagic areas soften, undergoing cystic degeneration.

While, as stated, the findings in the central nervous system are the most characteristic and the most constant, similar changes may occur in other organs of the body. It was not until 1919 that any special study appears to have been made of the effects of carbon monoxide poisoning on the heart.

In this year Zondek of the University of Berlin made careful studies of three cases of illuminating gas poisoning. All three recovered. In addition to the usual clinical studies careful records were kept of the pulse and the blood pressure and x-rays were made repeatedly to determine the size of the heart. As a result of his studies Zondek concluded that even in patients who recover from the effects of gas poisoning there is definite evidence of damage to the heart. He described a constant and diagnostic complex consisting of (a) a pronounced fall of blood pressure which usually lasts for about a week; (b) rapid pulse at the beginning, followed usually on the third or fourth day of convalescence by a pronounced slowing of the pulse to distinctly below the normal rate. This also lasts several days. (c) There often also develops irregularity of the heart, usually consisting of extrasystoles or a pronounced respiratory arrhythmia. His x-ray studies demonstrated (d) a definite dilatation of the heart following gas poisoning usually beginning very shortly after the poisoning, with a recovery from the dilatation after three or four days. The degree of dilatation appeared to vary depending upon the previous health of the heart. In one case, that of a thirty-year-old man, the dilatation did not appear until the third day and in this case at the end of two weeks the patient was still suffering from symptoms due to the "cardiac dilatation." Zondek speaks of this type of reaction as delayed dilatation.

In the same year H. Liebmann of the University of Munich confirmed the work of Zondek in a report in which he stated that he also had observed several cases with similar heart changes. He also reported the autopsy findings in a case of illuminating gas poisoning in which he found multiple hemorrhages in the brain substance and an interstitial and parenchymatous myocarditis which he felt was due to the effects of the gas poisoning. This investigator had failed to demonstrate changes in the heart muscle in experimental animals following carbon monoxide poisoning, but in this connection he remarked "nevertheless the case which I have just described must be considered proof that carbon monoxide poisoning exerts a direct toxic effect on the heart muscle which in severe cases manifests itself as an interstitial and parenchymatous myocarditis." Concerning failure to find these heart muscle changes more often at autopsy he states that with the methods of microscopic examination that are available the myocardial changes are of such nature that they can easily be overlooked and indeed can only be found after prolonged study with serial sections.

In 1920 G. Herzog reported his study of the pathology of illuminating gas poisoning before the Medical Society of Leipzig. He found as regards the heart, that eight out of ten autopsies of persons dead from gas poisoning showed evidence of damage to the heart muscle. He described the microscopic findings as being chiefly hemorrhagic necrosis and leucocytic infiltration. In one woman of thirty years who died three days after the poisoning there was a very pro-

nounced dilatation of the left side of the heart. One child who died twenty-three days after gas poisoning showed marked changes in the heart muscle.

In 1925 Gurich of the Pathologic Institute of the Hamburg-Eppendorf General Hospital again confirmed the work of these other investigators, adding additional cases of his own. In three of four fatal cases he found the characteristic heart lesions. He remarked on the occurrence of the minute hemorrhages in the heart muscle similar to those found in the brain. He further remarked on the great variation of the reactions and findings in individual cases. One person may manifest serious damage while another shows little or none of a permanent nature. For example, two brothers who died at the same time from gas poisoning. One showed the characteristic heart changes while the other failed to do so. This author believes from his studies that the gas acts as a direct poison to the heart muscle cells.

Concerning the ultimate changes in the hearts of persons who recover from gas poisoning we can of course have no information but Gurich believes from his studies that there probably remains some permanent damage with scar tissue formation in the heart.

In 1930 Martin Israelski and Ernst Lucas, working in Zondek's Clinic described two additional cases of illuminating gas poisoning both of which eventually recovered, in both of which x-ray examination showed evidence of hemorrhage into the lungs and in one of which there was a dilatation of the heart similar to that previously described by Zondek.

Howard W. Haggard in 1921, studying experimental carbon monoxide poisoning in animals concluded that there was no direct action of this poison on the nerves controlling the heart. It will be noted in this connection that the work of the German investigators above described indicated that the poisonous effect of gas on the heart was not on the nerves controlling the heartbeat but on the heart muscle itself.

In comparing the poisonous effect of pure carbon monoxide gas with that of illuminating gas Haggard found that the accessory toxic substances present in illuminating gas in addition to carbon monoxide caused more rapid asphyxiation than pure carbon monoxide, due to the fact that they stimulated the respiration, thereby hastening the development of respiratory fatigue and failure; but under the conditions of the experiment, he found no evidence that these accessory substances were in and of themselves directly or rapidly toxic.

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—*W. T. V.*